



GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS

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Daniel J. Hasselman, Stephanie A. Harmon, Amanda R. Matala, Andrew P. Matala, Steven J. Micheletti, and Shawn R. Narum

Columbia River Inter-Tribal Fish Commission (CRITFC), Hagerman, ID, 83332

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Executive Summary

This project combines four inter-related studies from the Accords Agreement that address the following current and future objectives:

Objective 1) discover and evaluate SNP markers in salmon and steelhead and other anadromous fishes. In the current year of this project we have continued our use of GT-seq protocols for SNP discovery. Our laboratory has designed SNP panels for five study species (Chinook salmon [*Oncorhynchus tshawytscha*] – 299 loci; Steelhead trout [*O. mykiss*] – 379 loci; Sockeye salmon [*O. nerka*] – 382 loci; Coho salmon [*O. kisutch*] – 257 loci; Pacific lamprey [*Entosphenus tridentata*] – 316 loci) and early development is ongoing for a sixth species (White Sturgeon [*Acipenser transmontanus*] – 117 loci). Expansion of the sockeye SNP panel (from 93 to 382 markers) is expected to provide necessary statistical power to perform single parent assignment analyses in Sockeye salmon while also improving genetic stock identification. The expanded panel for *O. mykiss* (from 269 to 379 markers) will improve resolution of genetic stock identification for steelhead in the mid-Columbia and lower Snake River. Further, markers associated with specific traits were added to GT-seq panels for *O. mykiss* to enable identification of premature (summer-run) or mature (winter-run) steelhead and fish with high thermal tolerance.

Objective 2) expand and create genetic baselines for multiple species including Chinook Salmon (*Oncorhynchus tshawytscha*), steelhead trout (*O. mykiss*), Sockeye Salmon and kokanee (*O. nerka*), Coho Salmon (*O. kisutch*) and Pacific Lamprey. Objective two of this project describes efforts to evaluate genetic diversity among populations that will inform managers in the areas of harvest monitoring, and conservation monitoring. Our approach involves the collection, analysis, interpretation and distribution of genotypic data. These data are being compiled as species-specific reference baselines for characterizing Chinook salmon, steelhead trout, and *O. nerka* population structure specific to the Columbia River Basin. The collaborative, inter-agency application of genetic stock identification (GSI) tools continues to provide invaluable monitoring capabilities to understand relative stock proportions in sport, commercial and tribal harvests, as well as monitoring of stock specific run-timing at Bonneville Dam, Lower Granite Dam and other fish weirs in the basin. Moreover, GSI is being used in concert with parentage based tagging (PBT; *O. tshawytscha*, *O. mykiss*, *O. kisutch*), providing the means to genetically assign individual fish to a hatchery broodstock-of-origin. PBT continues to be valuable for monitoring trends in hatchery production, harvest of hatchery fish, and population attributes of specific hatcheries (e.g., stray rates, survival/mortality, migratory behavior, hatchery/wild interactions). Major accomplishments in 2017 include expansion of our PBT baselines with the addition of several new hatcheries in the Columbia River Basin, both above and below Bonneville Dam. The GSI baseline for *O. nerka* was expanded from 93 SNP markers to 364 SNP markers. Secondly, the GSI baseline for steelhead trout was substantially expanded from 269 SNP markers to 372 SNP markers after screening thousands of RAD tags. Of this addition, 63 SNPs were specifically designed for their ability to differentiate between 7 smaller reporting groups within the larger MGILCS reporting group spanning the middle Columbia and lower Snake rivers. As a result, a large geographic region containing multiple sub-basins was resolved into individual sub-basins for accurate assignment of fish in GSI analyses. The expansion also included 69 SNPs that displayed significant associations with environmental variables in landscape genetic analyses. Some SNP were informative for both applications (i.e. differentiating MGILCS and landscape genetics). Lastly, the final 372 SNP markers include 6

newly designed markers in the GREB1 gene region, which is involved in maturation and has been shown to be highly informative for differentiating between summer-run and winter-run steelhead ecotypes.

Objective 3) implement GSI programs for mainstem Chinook salmon, Sockeye salmon, and steelhead fisheries. In this section, we first evaluate the accuracy of our PBT and GSI baselines for assigning Chinook salmon and steelhead to their hatchery brood (PBT) or reporting group (GSI) of origin. Results of the PBT testing suggest a high degree of accuracy for Chinook salmon (>99%) and steelhead (>99%) when the full suite of baselines that an individual could be assigned to are available. Moreover, our expanded SNP panels used for PBT assignments (Chinook: 298 SNPs; steelhead: 186 SNPs) provide more confidence in identifying the hatchery brood of origin, as reflected in elevated LOD-scores for both species. Results of the GSI testing also reveal accurate assignment of Chinook (Figure 1) and steelhead to reporting groups.

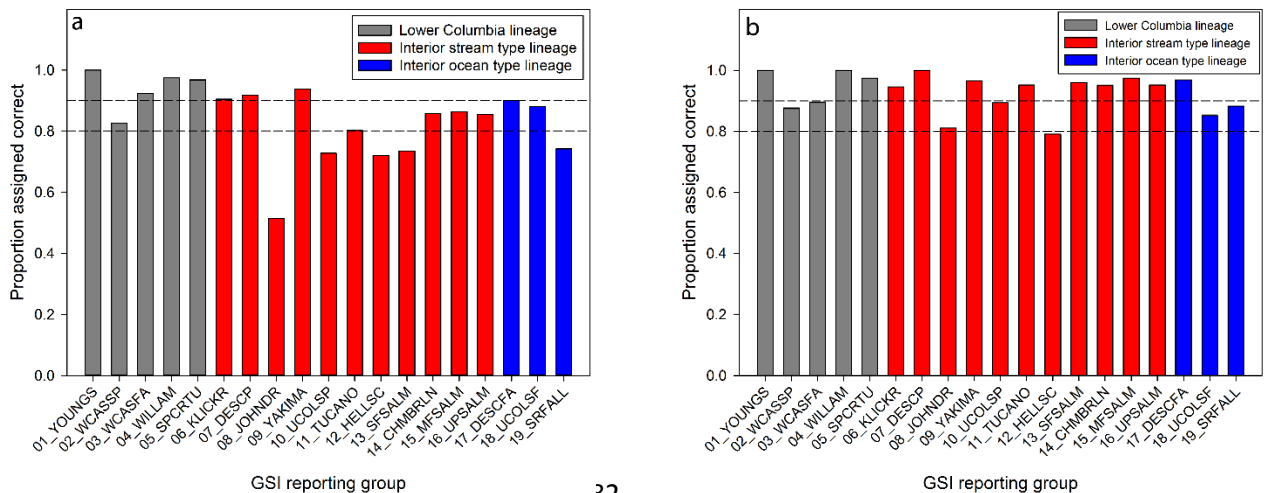


Figure 1: Proportion of Chinook salmon in leave-one-out tests that assigned correctly for each reporting group by lineage using a) all data, and b) $\geq 80\%$ self-assignment probability threshold. The dashed lines indicate 80% and 90% thresholds for correct assignment.

Upon completion of the accuracy testing, we used a combination of PBT and GSI analyses to determine stock composition of Chinook salmon harvested in 2016 in sport, commercial, and tribal fisheries in the mainstem Columbia River, and use GSI to estimate stock composition of sockeye salmon harvested above and below Bonneville Dam in commercial, sport, and tribal fisheries during the spring, summer, and fall management periods (Figure 2). We characterized the stock composition of a mark-selective sport fishery from the mouth of the Wind River (above Bonneville Dam) which has recently expanded its fishing boundary into the Columbia River mainstem. We detected PBT assignments to the Carson Hatchery (31%) representing 3- and 4-year old fish, and we anticipate that this fraction will increase as our PBT baseline for the Carson Hatchery will be able to assign 3-, 4-, and 5-year old fish from this fishery in 2017. We also observed that a large proportion (51%) of this fishery assigned to the 10_UCOLSP reporting group that includes Carson Hatchery, Walla Walla hatchery, Umatilla Hatchery, and upper Columbia River stocks (Figure 3).

Analysis of adipose-clipped Chinook salmon from multiple fishery mixtures in the spring management period (April to June 15th) identified relatively larger proportions of individuals that assigned via PBT to Snake River hatcheries. Chinook salmon from Snake River hatcheries comprised the largest component of each harvest, and accounted for 26-34% of fish harvested in Region B, and 37-50% of fish harvested in Region A from commercial, sport, and Test fisheries (Figure 4). These proportions are broadly consistent with the large proportion of hatchery origin Chinook salmon passing Bonneville Dam in 2016 that assigned via PBT to Snake River hatcheries (44%)

Limited sample sizes from adipose-intact commercial fisheries restricted comparisons of stock composition to the test fishery (n=38). GSI reporting groups of the interior stream-type lineage comprised the greatest fraction of adipose intact Chinook taken in the test fishery (29%), followed by assignments to the Lower Columbia lineage (21%), and PBT assignments to Snake River (13%) and Columbia River (13%) hatcheries (Figure 5).

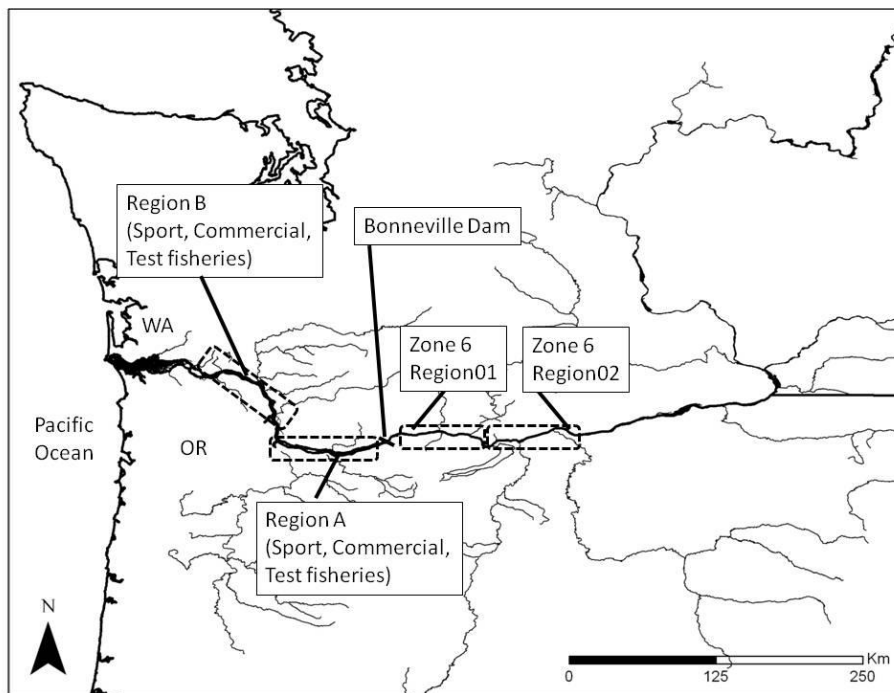


Figure 2: Sources of fishery mixtures in the lower Columbia River mainstem

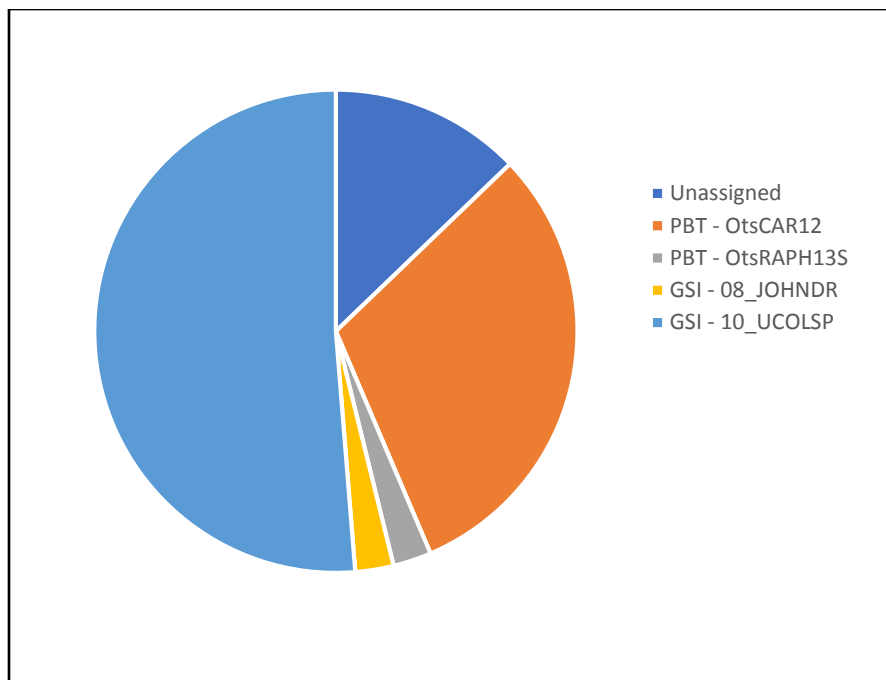


Figure 3: Genetic stock composition of the Wind River sport mark-selective harvest in 2016.

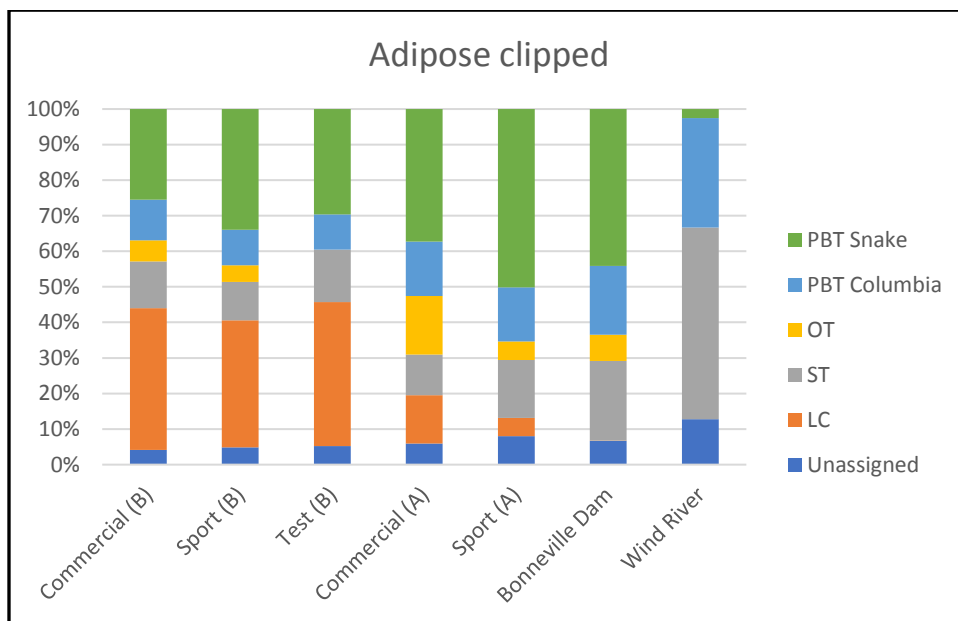


Figure 4: Stock composition of spring management period adipose-clipped Chinook salmon harvest mixtures collected in 2016. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

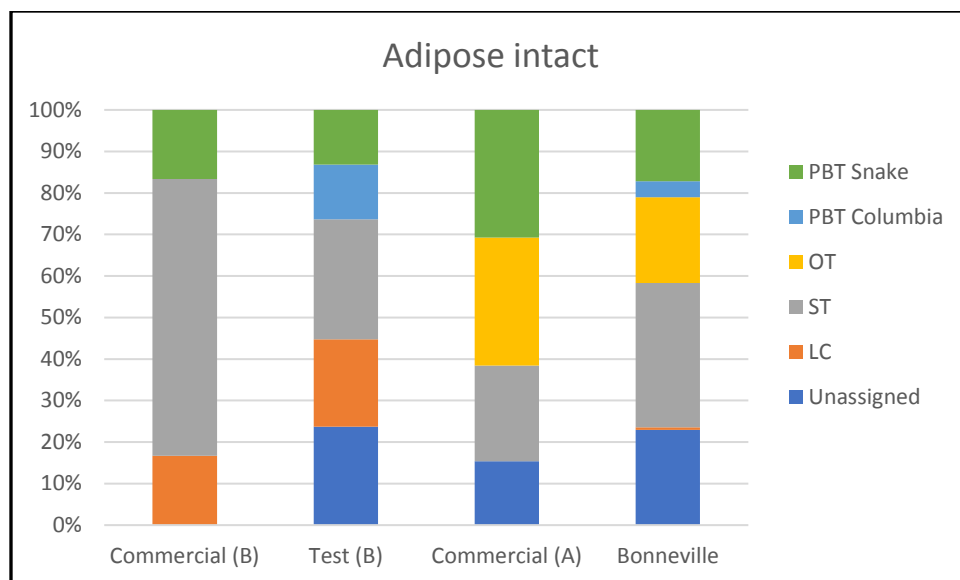


Figure 5: Stock composition of spring management period adipose-intact Chinook salmon harvest mixtures collected in 2016. ‘PBT Snake’ and ‘PBT Columbia’ include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

Analysis of Chinook salmon fisheries in the summer management period (June 16 – August 1) focused on understanding the proportion of upriver spring Chinook salmon stocks (ST) that were being harvested. Specifically, we sought to i) estimate the stock composition for sport and commercial fisheries below Bonneville Dam, ii) compare the stock composition of adipose-clipped vs. adipose-intact commercial fisheries below Bonneville Dam, and iii) characterize temporal changes in stock composition across the season.

We observed similar stock compositions for adipose-clipped Chinook salmon taken in Lower Columbia River sport and commercial fisheries from Region B (Figure 6). However, Chinook stocks from the OT lineage comprised a greater proportion of the sport harvest (59%) than the commercial fishery (39%) (Figure 6). While a similar proportion of adipose-clipped fish in commercial and sport fisheries from Region B assigned via PBT, a greater proportion of the sport harvest was assigned to Columbia River hatcheries (9%) than the commercial harvest (6%), and a greater proportion of the commercial harvest assigned to Snake River hatcheries (14%) than the sport harvest (6%) (Figure 6).

We detected a greater proportion of the LC lineage in the adipose-clipped sport harvest in Region B (18%) than in Region A (3%), but observed a greater proportion of the OT lineage in Region A (71%) vs. Region B (59%) (Figure 6). A similar proportion of fish in Region B (9%) assigned via PBT to Columbia River hatcheries vs. Region A (7%). However, a greater proportion of fish in Region A (11%) assigned via PBT to Snake River hatcheries vs. those from Region A (6%) (Figure 6).

Despite broad similarities in the stock composition of adipose intact commercial fisheries from Regions A and B, we observed that a greater proportion of fish in Region B (24%) assigned

to the LC lineage than those from Region A (12%), while a greater proportion of Region A fish (79%) assigned to the OT lineage than those in Region B (68%) (Figure 6).

We observed notable differences in the stock composition of adipose-clipped and adipose-intact Chinook salmon taken in the commercial fishery in Region B. As expected, a greater proportion of adipose-clipped fish assigned to Snake River hatcheries (14%) and Columbia River hatcheries (6%) than adipose-intact fish (2% and 0%, respectively). We also observed that a smaller proportion of the adipose-clipped commercial harvest was comprised of the OT lineage (39%) than the adipose-intact harvest (68%) (Figure 6). A greater proportion of adipose-clipped fish (35%) assigned to the LC lineage than adipose-intact fish (24%).

We observed differences in the stock composition of adipose-intact and adipose-clipped fish taken in the Zone 6 tribal harvests. As observed in other comparisons of adipose-clipped vs. adipose-intact fisheries, a greater proportion of adipose-clipped fish assigned to Columbia River hatcheries (9%) vs. adipose-intact fish (2%) (Figure 6). We also detected a greater proportion of adipose-intact fish assigned to the OT lineage (81%) than adipose-clipped fish (76%).

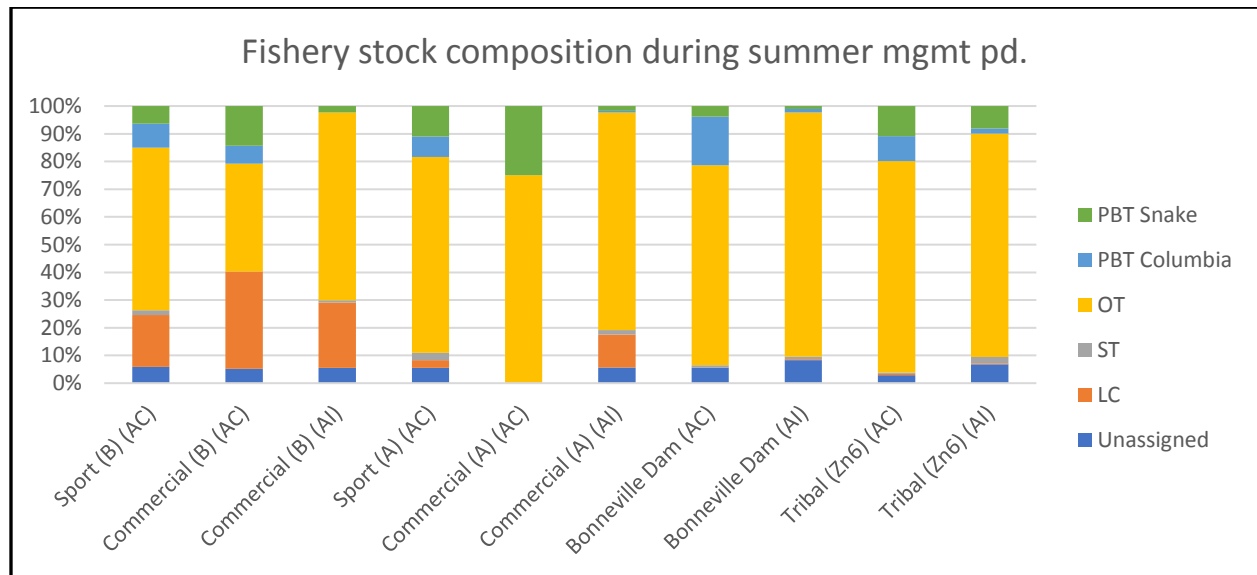


Figure 6: Stock composition of summer management period Chinook salmon fisheries and Bonneville Dam collected in 2016. ‘AC’ is adipose-clipped; ‘AI’ is adipose intact. ‘PBT Snake’ and ‘PBT Columbia’ include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

Small sample sizes limited our ability to compare changes in the % stock composition of the stream-type lineage of adipose-clipped vs. adipose-intact Chinook salmon over the course of the summer management period in the lower Columbia River commercial fishery relative to that passing Bonneville Dam. While we detected a decline in the proportion of ST lineage Chinook salmon for both adipose-clipped and adipose intact fish at Bonneville Dam, we also observed a modest increase in the proportion of the ST lineage for adipose-clipped fish at Bonneville Dam during statistical week 27 (Figure 7). However, our interpretations are limited by restricted sample sizes. As in previous years, meaningful comparisons are made challenging by the

absence of data continuity over the time series owing to fisheries closures and cessation of sampling at the Bonneville AFF in response to elevated water temperatures.

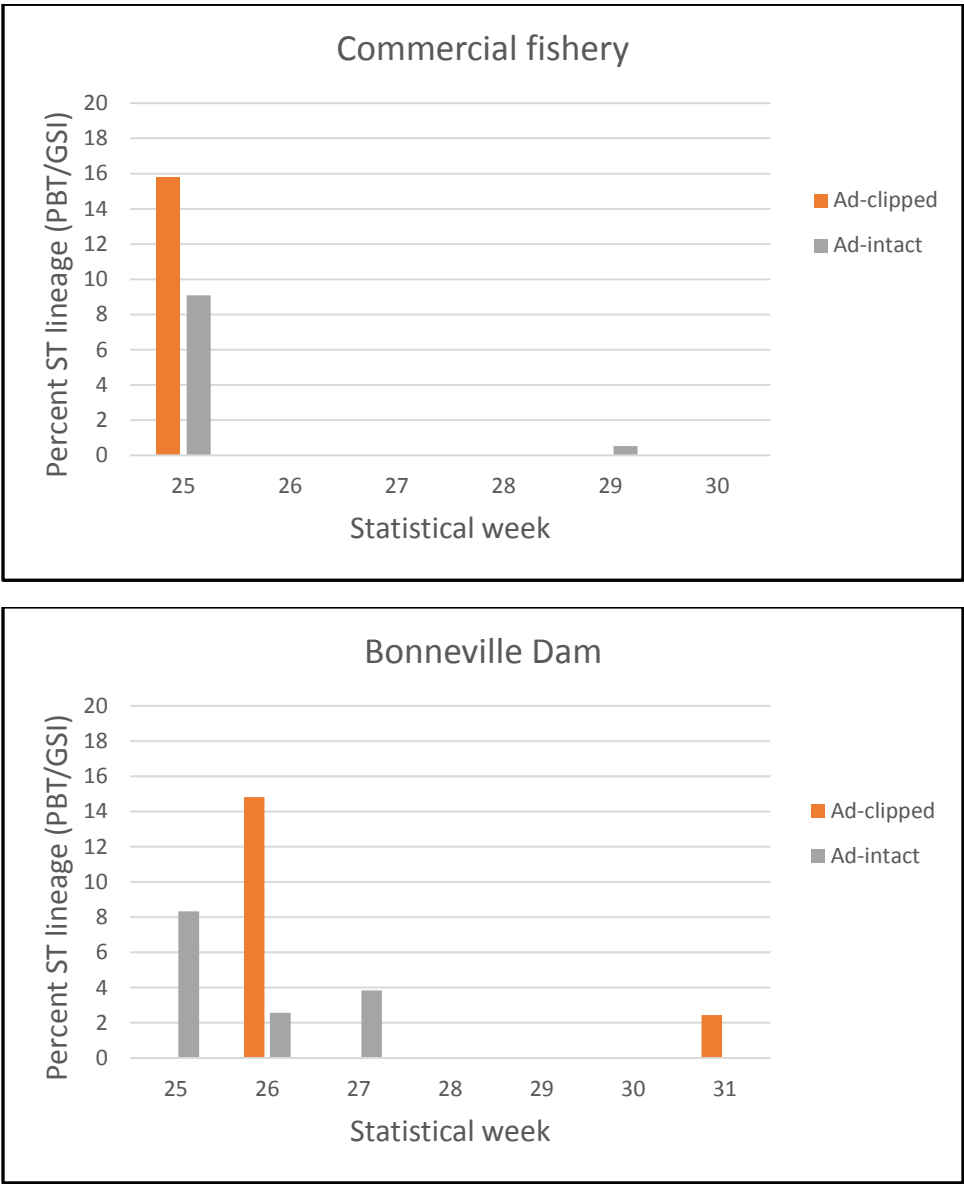


Figure 7: Temporal patterns from week 25-31 (2016) of the percent of Chinook salmon ST lineage in adipose-clipped and adipose intact mixture samples from the lower Columbia River commercial fishery (top panel) and Bonneville Dam (bottom panel) during the summer management period (June 16-August 31).

Analysis of stock composition of sockeye salmon fisheries included those from the lower Columbia River below Bonneville Dam in sport and commercial fisheries and the Zone 6 tribal fishery collected in 2016. The overall stock composition of sockeye salmon in these fisheries are shown in Table 1. The proportion of each sockeye salmon stock encountered at Bonneville Dam and in the Zone 6 tribal fishery varied over time (Figure 8).

Table 1: Relative stock composition for sockeye salmon taken in harvests and encountered at Bonneville Dam in 2016.

Mixture source	Stock proportion					
	Okanagan	Wenatchee	Redfish Lake	Lake Billy Chinook	Lake Wallowa	Okanagan
Commercial	54.08%	45.92%	-	-	-	54.08%
Sport	68.29%	30.67%	1.04%	-	-	68.29%
Zone 6	58.28%	40.67%	0.79%	0.18%	0.09%	58.28%
Total Harvest	58.81%	40.19%	0.77%	0.14%	0.09%	58.81%
Bonneville Dam	69.93%	29.14%	0.34%	0.33%	0.25%	69.93%

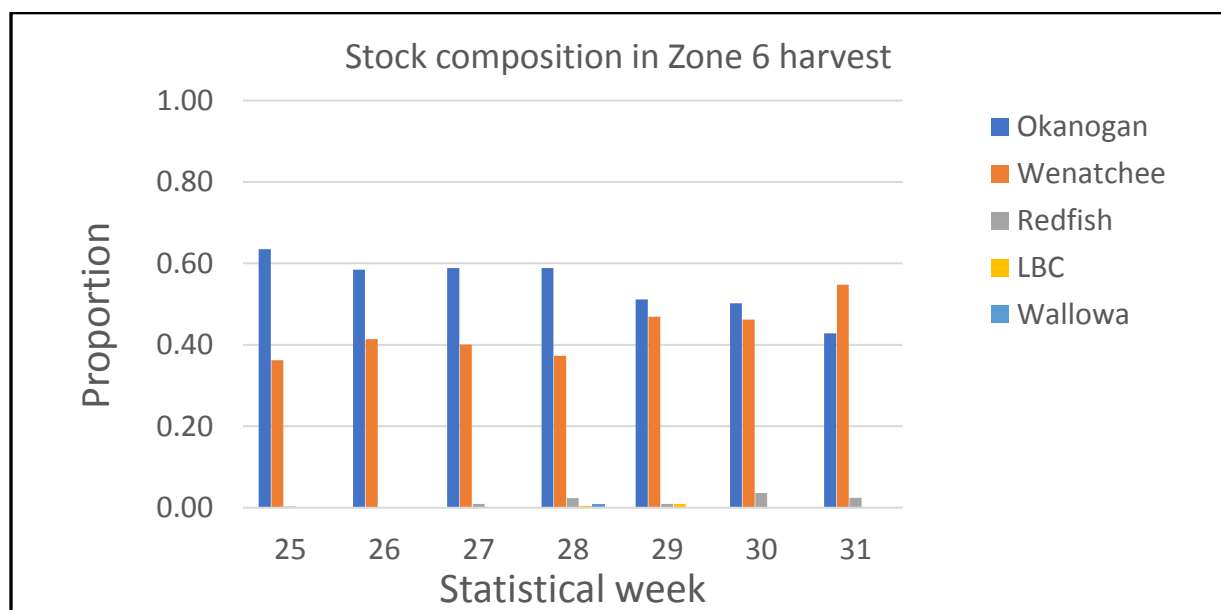
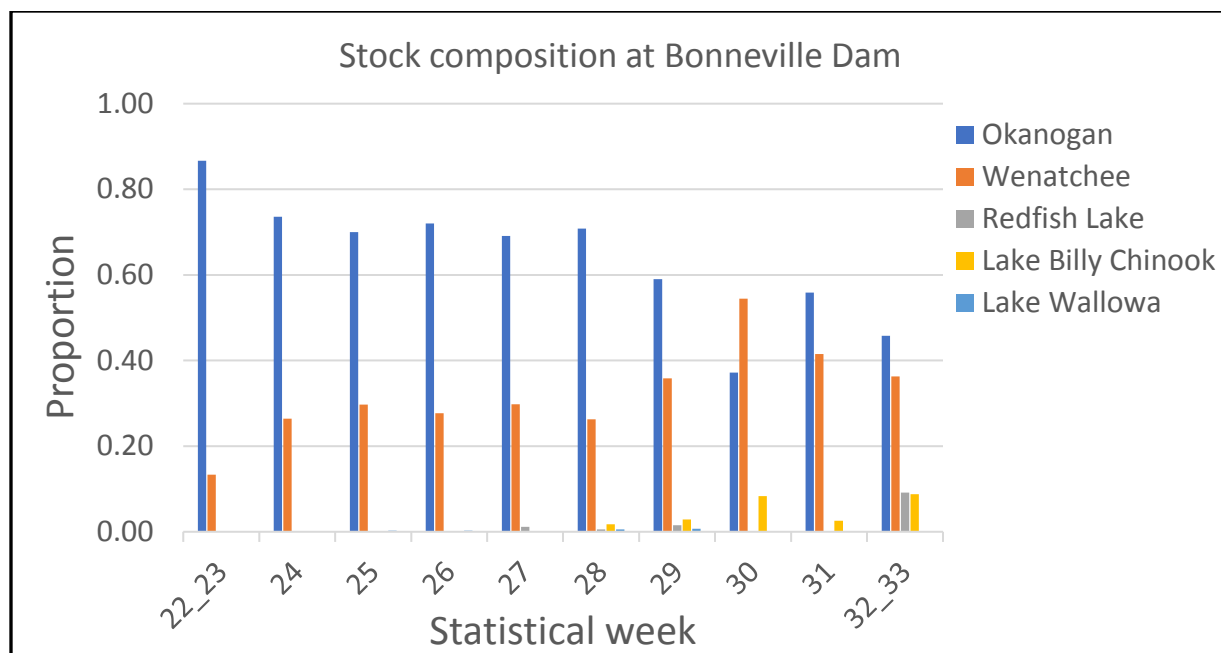


Figure 8: Stock composition of sockeye salmon at Bonneville Dam (top panel) and in the Zone 6 tribal harvest (bottom panel) across weekly strata.

Objective 4) Use PBT and GSI to estimate stock composition of fish passage at Bonneville Dam (steelhead, Sockeye salmon, and Chinook salmon). This section describes our efforts to determine the relative stock composition, abundance and migration run-timing distributions of hatchery and natural origin Chinook salmon, steelhead, and sockeye salmon passing Bonneville Dam. Fish were sampled as they migrated past Bonneville Dam. We sampled adult and jack Chinook and adult steelhead during the spring, summer, and fall management periods, and used a

combination of GSI and PBT to estimate run-timing distributions and relative abundance of hatchery and wild Chinook salmon and steelhead stocks in 2016.

There were 11 major (i.e., abundance >1000 fish) hatchery origin Chinook salmon stocks represented in the total estimate relative abundance (N=375,646) of hatchery Chinook salmon passing Bonneville Dam in 2016 (Figure 9). The majority of these (n=140,316) assigned to the 18_UCOLSF reporting group which is consistent with expectations of large returns of summer/fall Chinook salmon to Hanford Reach and surrounding areas. These estimates include relative abundance for PBT-assigned fish (adipose clipped and non-clipped) and adipose clipped fish that were assigned via GSI.

There were 12 major (i.e., abundance >1000 fish) Chinook salmon stocks represented in the total estimated relative abundance (N=390,320) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) Chinook salmon passing Bonneville Dam in 2016 (Figure 10). The majority of these (n=278,984) assigned to the 18_UCOLSF reporting group.

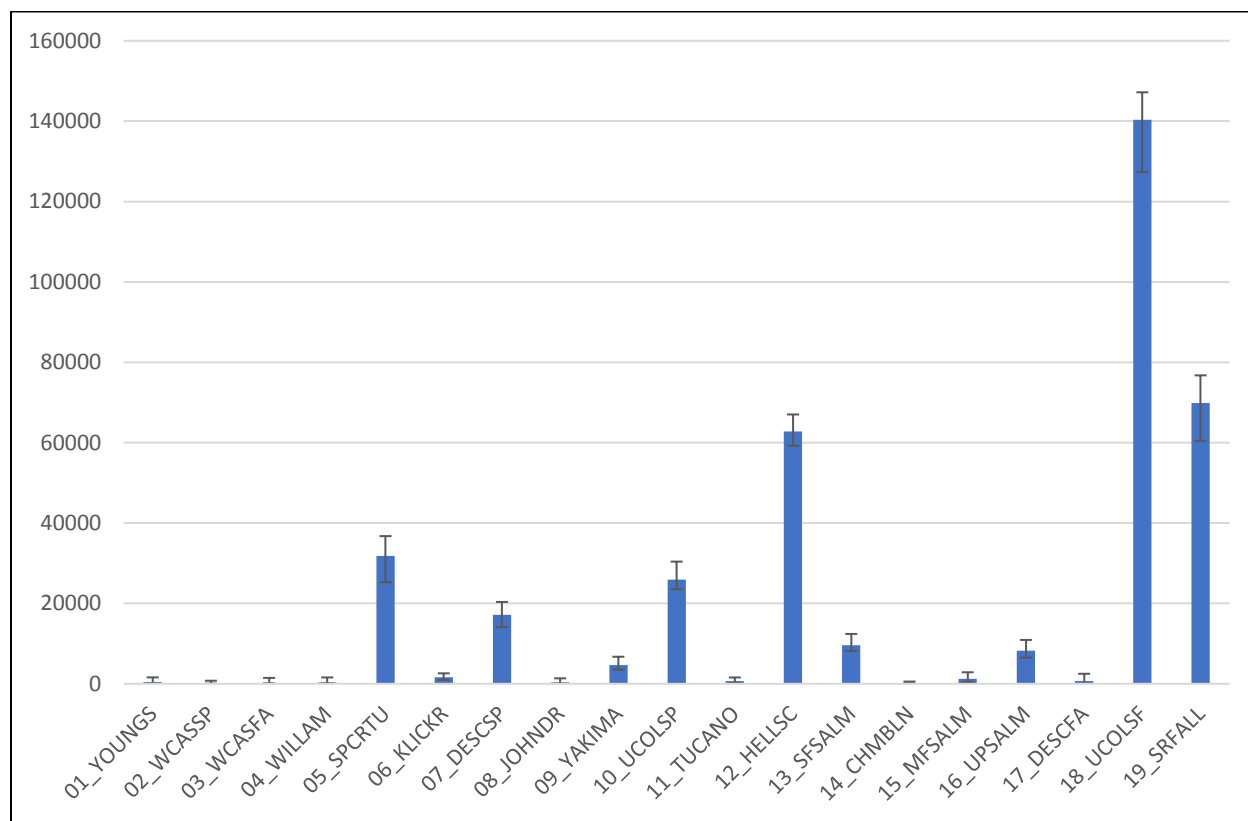


Figure 9: Relative abundance (± 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2016.

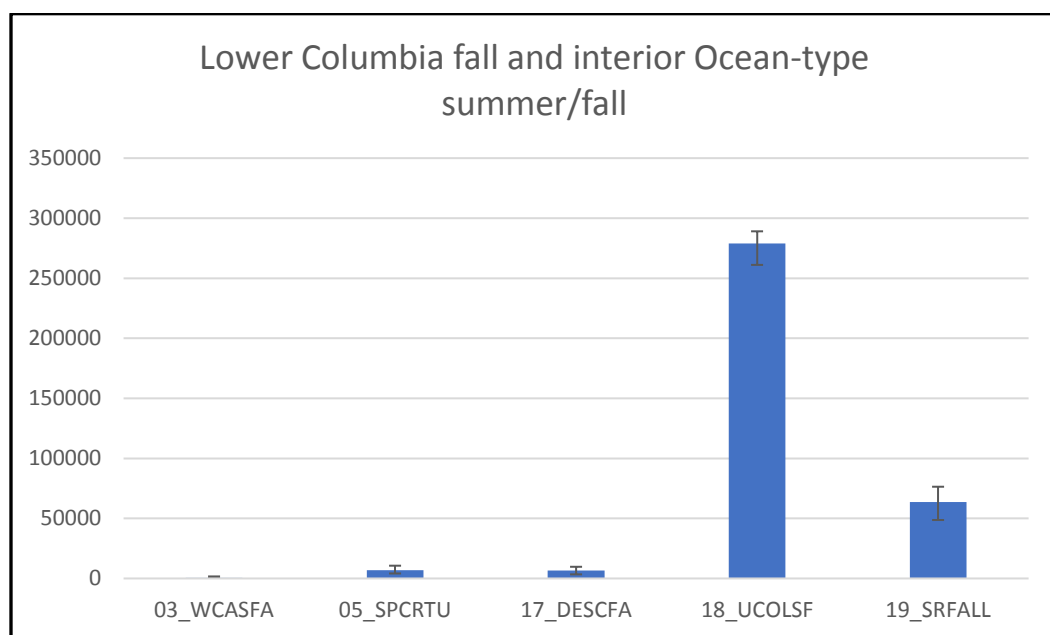
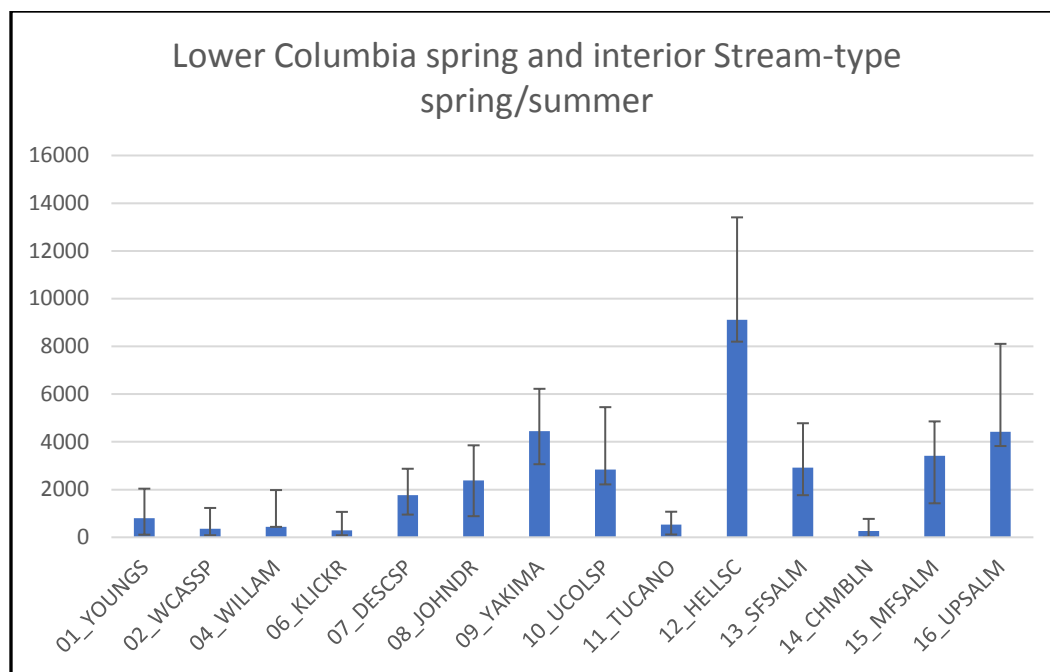


Figure 10: Relative abundance (\pm 95% CI) of natural origin (adipose non-clipped) Chinook sampled at Bonneville Dam in 2016 assigned to genetic stock of origin. Spring Chinook reporting groups (top panel), and fall Chinook reporting groups (bottom panel) are shown.

We identified five and eight major stocks (abundance > 1000) represented in the total estimated relative abundance of hatchery origin (N=142,412) and natural origin (N=39,218) steelhead passing Bonneville Dam in 2016, respectively (Figure 11). The 10_SFCLWR reporting group comprised the largest fraction of hatchery origin fish (51,452), whereas the 07_MGILCS reporting groups comprised the largest fraction of natural origin fish passing Bonneville Dam in 2016 (Figure 11).

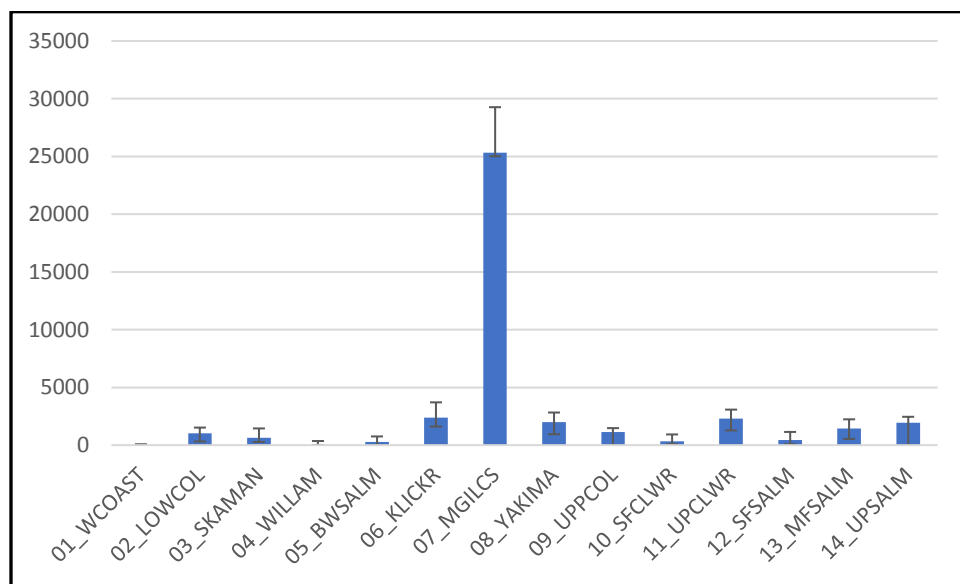
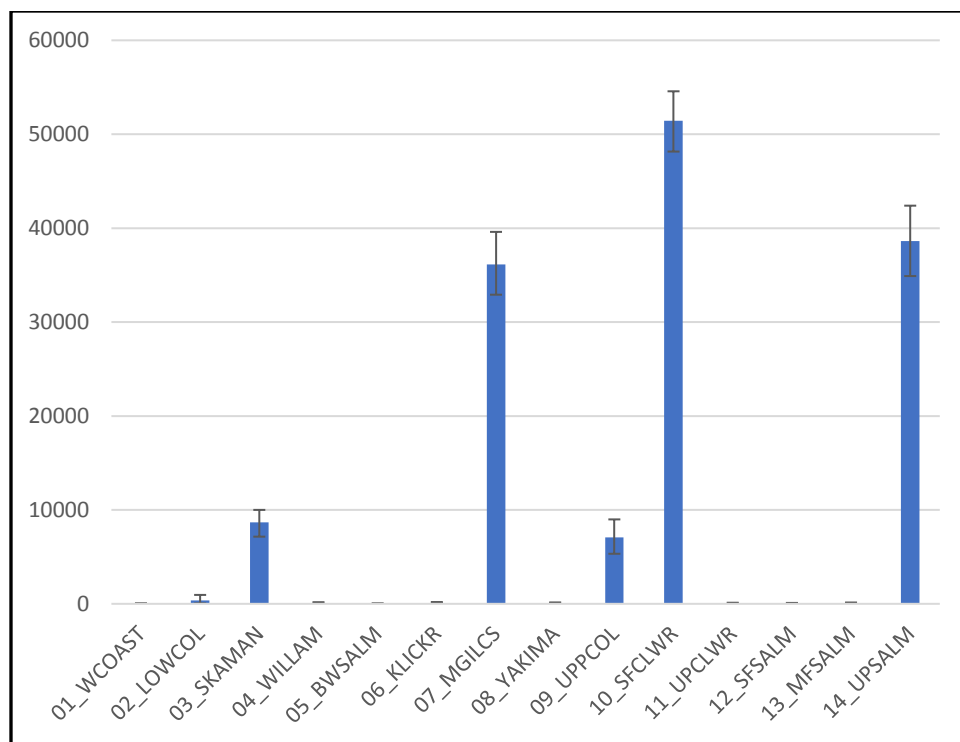


Figure 11: Relative abundance (\pm 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) (top panel), and natural origin (adipose non-clipped) steelhead (bottom panel) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2016.

The greatest proportion of sockeye salmon passing Bonneville Dam in 2016 assigned to the Okanogan stock (240,163), followed by the Wenatchee (99,037). and Snake River stock (7,919) (Figure 12). The Snake, Lake Billy Chinook, and Wallowa stocks all had estimated abundances < 1500 , but were based on relatively few genetic assignments ($n < 15$).

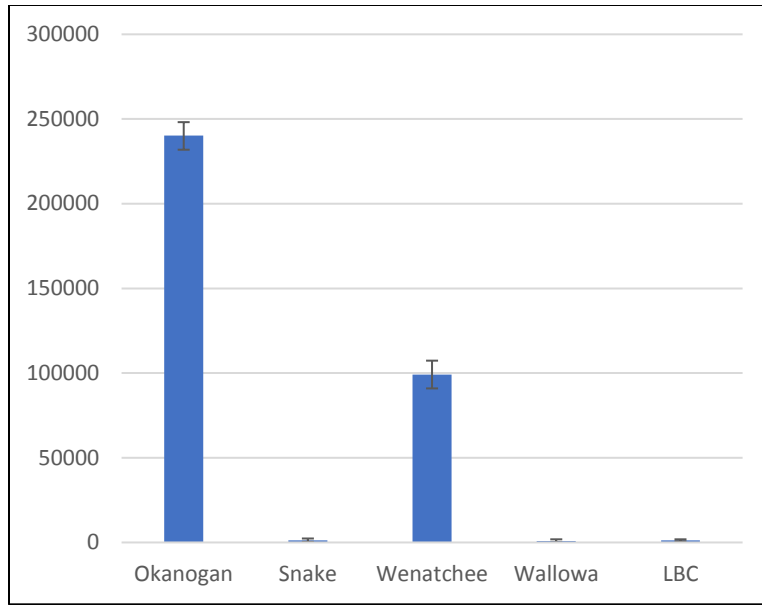


Figure 12: Relative abundance (\pm 95% CI) of sockeye salmon stocks sampled at Bonneville Dam in 2016.

While the run timing distributions of some hatchery origin and natural origin spring Chinook salmon stocks terminated within the spring management period, several spring Chinook salmon stocks extended well into the summer management period. The run timing distributions for hatchery origin and natural origin fall Chinook salmon stocks all had median dates on or after 8/27/16. For steelhead, we identified an early Skamania summer-run (median run-timing date 6/22/16), an intermediate run-timing category that contains most wild and hatchery steelhead stocks from the 09_UPPCOL, 07_MGILCS, and 14_UPPSALM reporting groups (median run-timing date range: 7/30/16 – 8/12/16), and a late run-timing category comprised of hatchery and natural origin stocks from the 10_SFCLWR reporting group with median run-timing dates after 08/25/16. Run timing distributions for sockeye salmon sampled at Bonneville Dam broadly overlapped in 2016, and we observed nearly identical run timing distributions for the Okanogan and Wenatchee stocks (median date: 6/21/16 and 6/22/16, respectively).

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Introduction

This project combines four inter-related studies from the Fish & Wildlife Program Accords that address the following current and future objectives: 1) discover and evaluate SNP markers in salmon, steelhead, and lamprey; 2) expand and create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho); 3) implement Genetic Stock Identification (GSI) sampling programs for mainstem Chinook salmon, sockeye salmon, and steelhead fisheries and 4) GSI of fish passing Bonneville Dam (salmon and steelhead). These four projects are highly related since SNP markers are needed to complete species-specific baselines, and these baselines are requisite to complete GSI. The results of these four objectives address needs for distinguishing specific stocks, determining genetic diversity, stock specific run timing, and estimating stock composition which can provide information for fisheries management.

Objective 1) SNP Discovery

One of the highest priorities in the full-scale implementation of SNPs for salmon genetics is the discovery and development of a sufficient number of markers to characterize population variability. These DNA sequence polymorphisms represent the most abundant variation in the genome of most organisms, and are spread throughout the entire genome at high density (Morin et al. 2004). Thus, SNPs can be discovered through sequencing known regions of DNA and converted to high throughput assays (e.g., Campbell and Narum 2008a), and more recently SNP discovery has become even more efficient for rapid identification of thousands of SNPs using genotyping-by-sequencing technology (e.g., Hess et al. 2013). Mutation rates, mutation models and error rates for SNPs are generally well understood, providing a foundation for estimating genetic divergence between populations. SNP markers offer a more cost-effective and less error-prone alternative to previous genetic marker technology such as microsatellite markers. Over the past several years, our lab has contributed to the increasing numbers of SNP markers that are available for salmonids and lampreys, and we have reached a point where rigorous stock composition and assessment goals for timely management of fisheries and highly accurate, precise stock assignments can be achieved using panels of SNP markers.

Objective 2) Baseline Expansion

Currently, genetic baselines of microsatellite markers are in place for Chinook salmon across the coastwide range (Seeb et al. 2007), steelhead (Blankenship et al. 2011), and *O. nerka* (including kokanee) in the interior Columbia River Basin. Despite large, representative sample sizes from many populations and high microsatellite allelic diversity, the resolution of specific stocks and populations in these baselines is limited in some cases. For example, Upper Columbia summer and upriver fall Chinook salmon in the Columbia River are closely related and remain impossible to distinguish even with a powerful set of 13 microsatellite markers. Several other closely related populations in the Chinook salmon baseline are similarly difficult to distinguish and thus have been pooled into a single reporting unit for GSI applications. In some cases (e.g., mainstem Columbia River Chinook fisheries) a finer level of stock discrimination is necessary to match data utilized by managers such as information provided by CWTs. Additional SNP loci will increase stock assignment reliability where greater resolution is desired. Given the difficulty and expense of inter-laboratory standardization, additional microsatellite markers are not the most efficient choice. In this regard, SNP markers are the preferred option for additional loci since they offer many beneficial characteristics that make them amenable to adding loci to existing baselines. Our two primary objectives for utilizing SNP baselines to monitor salmon species in the

Columbia River are 1) genetic stock identification (GSI) of natural-origin stocks, and 2) parentage based tagging (PBT), a large-scale, non-lethal tagging technology for monitoring and evaluating hatchery stocks.

Objectives 3 & 4) Genetic Stock Identification

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock applications (Narum et al. 2008b, Hess et al. 2011, Hess and Narum 2011). This study includes two GSI projects that will utilize genetic baselines: 1) GSI to provide information about harvest; and 2) GSI of fish passing Bonneville Dam.

This study includes GSI analysis of Chinook salmon and Sockeye salmon collected from commercial, recreational, and tribal fisheries in the Columbia River. Subsequent years of the study may include other species such as coho salmon. Implementation of GSI technology could make monitoring individual production units in mixed stock areas possible. Tissues will be sampled annually from fisheries with existing programs in place with Washington Department of Fish and Wildlife (WDFW), Oregon Department of Fish and Wildlife (ODFW), Yakama Nation Fisheries Program (YNFP) and Confederated Tribes of the Warm Springs of Oregon (CTWSRO). We plan to genotype representative samples from fisheries of primary interest. The GSI estimates may help fill information gaps on wild fish with a different resolution than can be estimated using methods such as CWTs.

The second application of GSI analysis in this study includes sampling unknown origin salmon and steelhead at Bonneville Dam for genetic analysis. Samples will be collected over the majority of the run on a weekly basis, and genetic baselines will be utilized to determine the stock composition of these runs. Few studies have been able to determine the extent of overlap among life history types of salmon and steelhead, but GSI of each life history type will allow us to determine the stock composition of the different runs through Bonneville Dam which can be compared to other methods such as using fish PIT tagged as juveniles. Population genetic methods and statistical assignment models have advanced dramatically in recent years, and estimating stock composition is now possible using either Bayesian or Maximum Likelihood methods (Anderson et al. 2008). Therefore, we plan to estimate stock composition of multiple species passing Bonneville Dam and provide this information on a timely basis to fisheries managers in the form of an annual report.

Finally, we continue to utilize a new genetic technology, parentage based tagging (PBT), in combination with GSI to help augment and refine our stock identification results. PBT is an efficient approach for mass-tagging of fish. The method is carried out by first genotyping a set of potential parents which then provides the opportunity to assign a set of genotyped offspring to their true parent pair. PBT is currently being utilized on a broad scale in the Columbia River Basin to tag all Snake River Chinook salmon and steelhead hatchery broodstocks (Steele et al. 2011) and we now have a baseline that includes most Chinook salmon and steelhead hatcheries located above Bonneville Dam. This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years. When parent pairs of a Snake River hatchery fish are identified with PBT, we can provide accurate information including age of the fish and the source hatchery in which its parents were spawned. We can now use PBT in

both Chinook salmon and steelhead GSI applications to identify all Snake River hatchery-origin fish, and then we estimate stock-of-origin of all other hatchery fish that were not assigned with PBT (i.e. non-Snake River hatchery-origin) and all wild fish using GSI. In this way PBT and GSI are complimentary, and using them in combination takes full advantage of the strengths of each method, while resolving or minimizing limitations. Exogenous stock transfers by hatcheries have made hatchery-origin fish challenging to assign with GSI and represents a main limitation that is addressed with PBT. Applications of PBT have been initiated in other species such as Pacific lamprey, and are being used to monitor translocations of lamprey throughout the interior of the Columbia River.

Report Structure

This report is divided into four sections, one for each of the objectives of the study. The first section reports on SNP discovery efforts and the second section on genotyping SNP markers in Chinook salmon, steelhead, and *O. nerka* to create genetic baselines. The third section contains stock composition estimates of Chinook salmon and Sockeye salmon sampled in mainstem fisheries in 2016. The fourth section includes analysis of run-timing distributions and estimated abundance of adult Chinook salmon, Sockeye salmon, and steelhead stocks migrating over Bonneville Dam in 2016.

Section 1: SNP Discovery

Introduction

Population genetic studies examine variation within the genomes of individuals in order to gain insights into the nature of those populations. For instance, genetic similarities among groups of individuals can indicate relatedness, recent population collapse, or barriers to migration. In the context of salmon conservation, population genetics can answer important questions directly related to fisheries management such as stock exploitation rates, effective population size, and rate of return. Other demographic information such as stock abundance estimates can also be made through analysis of samples taken from fish as they enter the Columbia River through genetic stock identification (GSI). These studies require genotype data from a suitably large and informative set of genetic markers for analysis. Likewise, the number of genotyped individuals must be suitably large to provide accurate results.

Next generation sequencing instruments can provide both a means to identify genetic variation and provide a platform for high-throughput sequencing. Methods such as restriction-site associated DNA sequencing (RAD-seq: <https://www.monitoringmethods.org/Method/Details/4144>) can be used to identify and genotype thousands of single nucleotide polymorphisms (SNPs) within and among study populations. The most informative SNP loci are chosen for inclusion in high throughput genotyping panels. Genotyping in Thousands by sequencing (GT-seq: <https://www.monitoringresources.org/Document/Method/Details/5446>) is a high throughput method that uses Illumina sequencers to rapidly genotype thousands of individual samples at hundreds of loci for less than ¼ the cost of previously used TaqMan assays (Campbell et al. 2015). Following the development of GT-seq, our laboratory has designed panels for 5 study species (Chinook salmon [*O. tshawytscha*] – 299 loci; Steelhead trout [*O. mykiss*] – 379 loci; Sockeye salmon [*O. nerka*] – 382 loci; Coho salmon [*O. kisutch*] – 257 loci; Pacific lamprey [*E. tridentata*] – 316 loci) and early development is ongoing for a 6th species (White Sturgeon [*A. transmontanus*] – 117 loci). All of these GT-seq panels have been designed to a maximum of 75bp to allow for inexpensive sequencing runs on Illumina NextSeq 500. Recent additions were made to increase GT-seq panels for *O. mykiss* to 379 SNPs (Table 1) and *O. nerka* to 382 SNPs (Table 2). The expanded panel for Sockeye salmon is expected to provide necessary statistical power to perform single parent assignment analyses in Sockeye salmon while also improving genetic stock identification. The expanded panel for *O. mykiss* will improve resolution of genetic stock identification for steelhead in the mid-Columbia and lower Snake. Further, markers associated with specific traits were added to GT-seq panels for *O. mykiss* to enable identification of premature (summer-run) or mature (winter-run) steelhead (Hess et al. 2016), and fish with high thermal tolerance (Chen et al. 2017).

Methods

For new SNP loci added to panels, the program Primer3 (Rozen and Skaletsky 2000) was used to design primers flanking the target SNP locus for inclusion in existing GT-seq panels. (GT-seq: <https://www.monitoringresources.org/Document/Method/Details/5446>) Parameters used for primer design are as follows (product size range: 50-80 bases, optimal annealing temperature: 60°C, primer size range: 18-24 bases, optimal GC content: 50%). The designed primers were then modified by including the Illumina sequencing primer sites. The primers were ordered from IDT (Integrated DNA technologies) at a concentration of 200µM at the 25nmole synthesis scale. Testing was done by combining primers from previous loci for each species that already worked for GT-seq with the newly designed primers. These new primer pools were then used to create test libraries containing 96 samples using the GT-seq protocol (Campbell et al. 2015). Test libraries were “spiked” into an Illumina HiSeq lane with another sequencing library such that each test library produced about 10 million reads of data for analysis. Since the test library uses only a small percentage of the total reads on the flow cell the new library can be sequenced very cheaply. The sequencing reads were analyzed for the presence of significant numbers of hetero-dimers produced in multiplex PCR using custom perl scripts (<https://github.com/GTseq/GTseq-Pipeline/>). Primers producing large numbers of sequencing artifact reads through primer hetero-dimer interactions were flagged and omitted from the next primer mix. Following this step the primer mix was used for full scale genotyping using GT-seq libraries containing up to 5,000 samples for a NextSeq flow cell.

Results

GT-seq primer pools are being used for all high throughput genotyping projects for 5 target species (Steelhead [*O. mykiss*] – 379 SNP loci including a sex determination marker, Chinook [*O. tshawytscha*]-299 SNP loci including a sex determination marker, Coho [*O. kisutch*]-257 SNP loci, Sockeye [*O. nerka*]-382 SNP loci, and Pacific Lamprey [*E. tridentata*]-308 SNP loci). The remaining primer pools remain unchanged from last year’s report but an additional SNPs from Pool-seq data will be under development in the coming year (Paired-end data assemblies, primer design, and testing). Our GT-seq panels have been used to genotype over 125,000 samples as of Dec. 8th in the 2017 calendar year (Figure 1).

SAMPLES GENOTYPED BY GT-SEQ IN 2017 (TOTAL: 151,884)

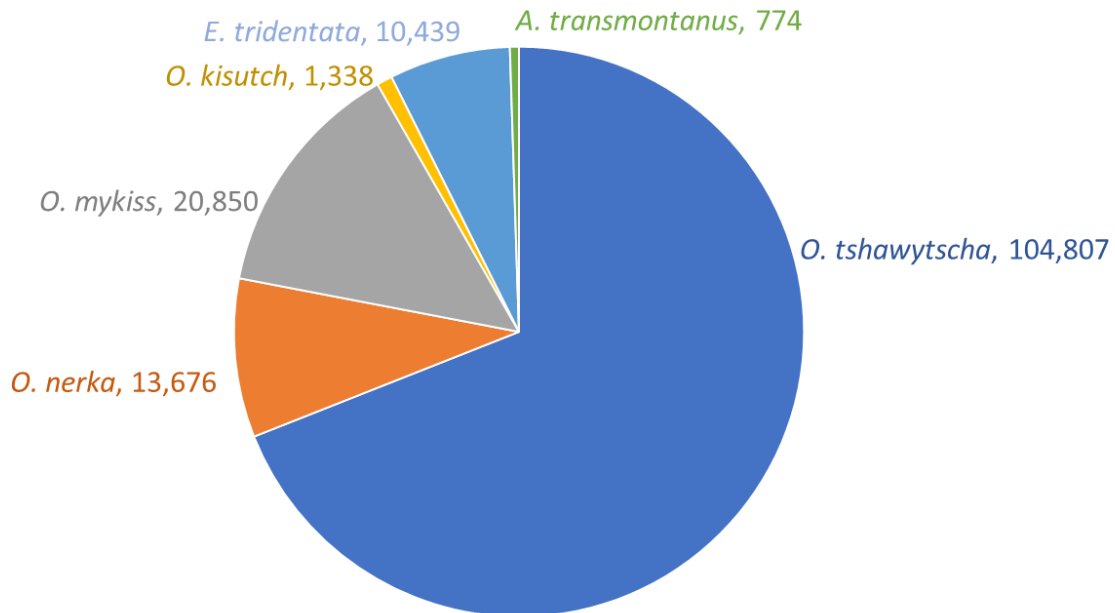


Figure 1: Summary of Columbia River fish samples genotyped using GT-seq in calendar year 2017.

Discussion

The GT-seq genotyping method has allowed for the genotyping of more samples in less time at more loci and at significantly cheaper cost than our previously used method (TaqMan genotyping assays). The total number of samples genotyped using this method has continued to increase from previous years but is expected to stay at a similar level in coming years of this project. The inclusion of more loci afforded by this method has also allowed for improved capabilities such as greater ability to discriminate between reporting groups in GSI and single parent assignments in parentage based tagging (PBT) projects. Similarly, we can now take advantage of genetic markers associated with physical and behavioral traits of our study species by including them in our high-throughput panels. An example of this is our ability to distinguish between summer and winter run steelhead by including two SNP loci found to be highly associated with run timing by Hess et al. 2016.

Expansion of our GT-seq panel for Sockeye salmon is also projected to greatly improve research capabilities in that species. The expanded panel contains 382 loci compared to the previous 93 loci, which should be sufficient for differentiation of Columbia River populations, but also provide statistical power for parentage analysis in this species.

In conclusion, the GT-seq method continues to produce quality genotyping data at a fraction of the cost of previous TaqMan genotyping assays. The technique uses only general laboratory instrumentation (Thermal cyclers, plate centrifuges, quantitative PCR instrument) for library preparation and the Illumina sequencers (either HiSeq or NextSeq500) can be used as high throughput genotyping platforms while maintaining utility for other sequencing studies (whole-genome shotgun, RAD-seq, transcriptome sequencing, synthetic long read, etc.). This is a key feature of the technique since it allows the multipurpose functionality of the laboratory without investment in specialized equipment. Overall, GT-seq is a valuable tool for conservation genetics studies allowing vastly improved statistical power, higher throughput, and prediction of heritable traits at a lower cost.

References

- Campbell NR, Harmon SA, Narum SR (2015) Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* **15**, 855-867.
- Chen, Z., A.P. Farrell, A. Matala, S.R. Narum. *In press*. Mechanisms of thermal adaptation and evolutionary potential in conspecific populations to changing environments. *Molecular Ecology*.
- Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proceedings of the Royal Society B*. **283**, 20153064.
- Rozen S, Skaletsky H. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, editors. *Bioinformatics Methods and Protocols*. Totowa, NJ: Humana Press p. 365-386.

802 Table 1: *Oncorhynchus mykiss* GT-seq panel (379 loci including one sex determining marker). This table contains the primer sequences, in-silico probe sequences for each allele, and correction values for each allele for each
803 SNP locus included in the *O. mykiss* GT-seq primer pool. Each forward primer is modified with a 5' ‘small RNA’ sequencing primer site (CGACAGGTTTCAGAGTTCTACAGTCCGACGATC) and each reverse primer is
804 modified with a 5' standard paired-end sequencing primer site (GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT).

Assay	A1	A2	Forward primer	Reverse primer	A1-Probe	A2-Probe	Allele Corrections
M09AAC.055	C	T	GTCTCCGACGTGTGGCT	TGGAACGAACTGAGAACATAAGG	ACCTCCACGCTGTCC	ACCTCCACACTGTCC	0,0
M09AAD.076	T	C	ACTGTTACCACTCTCTATCAACCT	GGGTCCAGGAGGTTTTTAACAACAT	CACCAACCACTGGTGAA	CCAACCGCTGGTGAA	0,0
M09AAE.082	T	G	CTATGTGCAGTGCCTTCTCA	GGCTTACAAGTATGCATGACTAGCT	AGGTTGTTTTACAAATTTAA	AGGTTGTTTTACACATTTAA	0,0
M09AAJ.163	G	A	TCCCATGGCCCTTACTCTATCAA	TTGAGGTGTATGTTGAAAAGTAAACTT	AACAAAGTGAAAGTGCTCTTA	CAAAGTGAAAGTGCTTTTA	0,0
Ocl_gshpx-357	T	G	GAGATCCTGAGGTCCTGAAGTAT	AAGTGAAATTTGGGCTCAAAGC	ATCCGTCCAGGAAATG	TCCGTCCCGAAATG	0,0
OMGH1PROM1-SNP1	A	T	TCAAACGTGATTTGATGGAAACAACAT	AGGACAATTCTAAGTGACCTCAAACG	TAGTGTTCACTGACTTCA	TAGTGTAACATGACTTCA	0,0
OMS00002	A	C	TTTGATTTGATTTGTATCTGCTTCTT	CCAACATGGCTCACACAAAA	TGTTTTGCAGCGCTC	TGTTTTGCACGCGCT	0,0
OMS00003	T	G	GTGCCACTGATGAGGATGAGATCA	GTAATAAGCCCTTTTGTGAGGAAAACTAAT	CTTACTGTCGACATTTTA	TACTGTCGCCATTTTA	0,0
OMS00006	T	C	TCCACGTAGGACATAGTTTGAGCTA	TGTGGTGCATGTTTGCCTAC	CACCTACAAATACAAAATT	CTTACAAATGCAAAATT	0,0
OMS00008	A	T	CCCTTTAAGGAGGATTTTAAATATGTGAGATAGAA	GGATACAGCGTTTTGGAATGAAACT	CTTCAAATATCCATAATTATATC	TCAAATATCCATAATAATATC	0,0
OMS00013	A	G	GCCTTTGTTCTCCTTGGTGGTTA	AGAAAAGTGTGGACTGAGGTTGAG	CTTCTTTTCCCTTGCTACTC	CTTTTCCCTCGCTACTC	0,0
OMS00014	T	C	CTTACACACAAGGGCTTCATTCTG	GATGTCTCTGGGTGGTTGTCA	TGATTTGATGAATTAAACTTC	TTGATGAATTGAACTTC	0,0
OMS00015	A	T	TCAGACCCTATTTTGGCACAAGT	GTCTAACTGATCCCACTTCTGCAT	CAAGTCACACTTTTAAATGAA	CAAGTCACACTTATAATGAA	0,0
OMS00017	A	G	ATTAAGTTTCATACAAAAGTTTCATCATAAATATTTTCCTTT	GGAGAACAAAGGGAAAGAGAAGACA	TAGACCTCGGTGCTGTAG	CCTCGGCGCTGTAG	0,0
OMS00018	T	G	AGAGTACATGTGTGGCTGCAA	GTCATAAATCAACACAATTATCTTCTTCACAGAA	AACCACATAATTATAATTC	CCACATAAATCATAATTC	0,0
OMS00024	T	G	CACATACAACCATCACCTTCTCTAA	AGCATTGAGCGAAATTACCAAGAGT	AA[AC]CCCAATTTTAC	AA[CA]CCCAATTTTAC	0,0
OMS00030	T	G	CCTCGTGACTACAGACTATACAAC	GATCTGATCGGTCGGGAGAGA	ATGAGGGTCCCTATACAGG	ATGAGGGTCCCTCTACAGG	0,0
OMS00039	A	G	GTCAGTACTGTGTGTGTCTGTGT	CCATCTACATTGTGAGCAGTGTGA	GTACGTGTCTGTGACC	GTGCGTGTCTGTGACC	0,0
OMS00041	G	C	GATTCTGTTCCATCTCTTTCTGTCA	AAACATAAAAAAGGCATGAAGGTGTC	CCACTCTATGCCTGCCCT	CACCTCTATGCCTGCCCT	0,0
OMS00048	T	C	GGAAGAGCTGGAGAACAACTG	TGCGATTGACAGAGGCTTTCTTT	CAGCTAAACTCAGCAAAA	AGCTAAACTCGGCAAAA	0,0
OMS00052	T	G	TGCGTTTTTTCATCCCAATCATTAC	GGCATCAGGCTCTTCTTCT	CTTCTTTTGAGAATAAT	CCTTTTGCGAATAAT	0,0
OMS00053	T	C	GGAGCCAGGTCAAGGTGATC	GGATGTCTGGTGTGGCTGTAAA	ATTTATATGTATCAATCA	ATTTATACGTATCAATCA	0,0
OMS00056	T	C	TCAGGAAGTAAACTGAAAATTCCAATGTATGA	CCCCAACCATGTCTGTTATTGAAC	TAGCTTGACCAAAATAGCA	CTTGACCGAATAGCA	0,0
OMS00057	T	G	GAGAAAGGGAGCATGAGACAGAG	GTTGGGCTCCGGTACGAT	CTCCACAGAACCTTG	CTCCACAGCACCTTG	0,0
OMS00058	A	G	GTGACATTTGGAGCCACTGC	GCTAGGAGACAGAGGGTGAAAG	CAACACTTTGTACCCCTC	CACTTTGCACCCCTC	0,0
OMS00061	T	C	AAGTGGAGCTGACCTGTTG	GCTGATGGAGCTGACAGTTAAT	CATTGCACTTTACAGACTT	TGCCATTTGACAGACTT	0,0
OMS00062	T	C	ACCCTGGGAAGGCTACTGTAC	TGAACAGAGATCTGGAGATTGGAT	TTGACCAGCAGATGGTGTA	ACCAGCAGGTGGTGTA	0,0
OMS00064	T	G	GTGGATATGTAGTTCGATGGAACAGT	TTTACAACAATCTCTTTTAATAAAAATATAGCCACTTAT	CAGGCAACATTTTATATAACTA	CAGGCAACATTTTATCTAACTA	0,0
OMS00068	A	G	GCCTAACTGGACAACATTTTAAAGATGA	GGCAGTTGAGCATTTTGGGATATT	AATATGCCTCCTTCGTCTC	TATGCCTCCTCGTCTC	0,0
OMS00070	T	C	CGTTCTCGGGACAGT	GTTTCTCTCAGTCCACAGATCT	CAAAATACGGAAATGCAG	AAATACGGGAATGCAG	0,0
OMS00071	A	G	CCGGAGTGACCTCACATTTGG	GCATCGTACAGTTCACCTACCT	CTGTTTGAGCTTTTTCT	TTGTTTGAGCCTTTTTCT	0,0
OMS00072	A	G	GTGGGAGAGCTCGTCTATGG	ACAACAGGTCAATTGGATGTGATCAG	TAGAAGGTCCATGTATCTC	AAGGTCCATGCATCTC	0,0
OMS00074	T	G	CCTGTTTATTATCTAAACAGTCTTTAAAAAT	AACTTAATTTAGCAAAACAATGTCTGAACAGAA	TGAAACAAAACAATGTTTCC	AAACAAAACAGATGTTCC	0,0
OMS00077	C	G	AATACCATCTTGAGCTCATTAGTAATTATTCAA	CCAGACTTTACACACTCTTGACTGA	TTCCGGTGGTGAAGTT	CCGGTGTGAAGTT	0,0
OMS00078	T	C	GAGGGAAGCAGCCATAAACAGAATA	GTCTCACTATGGTCCATATCTGTGTAGA	TTCACATGCATAAGAGTG	TCACATGCATGAGAGTG	0,0
OMS00079	T	C	GTAACATTATGAATCTATCAGTTTCCTAGCT	ACCTGCAACGTTAGAGCTGTTTATT	CTACTTTTCACAGTAACACAG	CTACTTTTCACAGTGACACAG	0,0
OMS00087	A	G	GCAAAATTCACCTTAACAGTGGTTT	GATTTGATGTGTGTGTTACCTCTCTA	GTTA[CA]AACTGACAAAGTGTG	GTTA[CA]AGCTGACAAAGTGT	0,0
OMS00089	A	G	GCACCATTTGAATAAAAAATCTGCTTGT	GCAACCAATTCATATTAAGCAGATGAT	ATGAATCCCAATAAGAAC	AATCCAAACAAGAAC	0,0
OMS00090	T	C	AGGGCACAACACCACTCTAAAT	TCGAAAAGCACAACCACTCTGCTCAAT	ACAACCAACAAGATT	AACCAACTCAAGATT	0,0
OMS00092	A	C	TCTCCAGGTGTATCTTGAGAAGGT	AGGGTTCACACAGGGAAGATATCAT	CAGCTGAGAATAGGTTT	AGCTGAGAAGAGGTTT	0,0
OMS00095	A	T	CTCCAATGGCTGTCAACAATTAATATAAGAC	GTGTGCTGGTCTCTTTTATTCTCA	AGGCAACTATATATTTTTT	AGGCAACTATATATTTTTT	1.5,0
OMS00096	T	G	CATGAGAATGGATCAGTCTCCACAA	GATGAAATCTGAAATGTGTTGACACTACAG	AAAGAGGAAGAGTCTCG	AAAGAGGAAGCGTCTCG	0,0
OMS00101	A	G	GCGTGTCTGGGTCAGTTAAATA	GTGCAATCCAACCTATTAGTAGATATGCT	CTTAGTAGCCTTATAGAAAAG	CTAGTAGCCTTACAGAAAAG	0,0
OMS00103	A	T	GAGATCACTGTAGGATTGGCTGTTT	CCTCAGAGCAGCTCACAATGGCATC	CTCCACAGTAATTTTTTTTT	CCACAGTAATTATTTTTT	0,0
OMS00105	T	G	ACATTTGAAGTCAGTATGGGTGTGAG	GAACCTCACACAGTACTAAATGCA	CTGCTATTCAAAATTGCT	CTGCTATTACATTGCT	0,0
OMS00106	T	G	CGGTAGCATTCTTGAGGAAGCTT	TTTCCAACAGATGGCAGAATCTCT	TCTGATGGAACCTTTC	TGATGGCAACTTTC	0,0
OMS00111	T	C	CATGCGGACCTGCATAGCT	GCTTAGCCATTGACAGAGCATATCA	CAACCAGACTACCATT	AACCAGACTGCCATT	0,0
OMS00112	A	T	TGGCAGCAAAAGGGATGCA	TCCTGAGCAACCACTCAACATT	CCGGTTTCAAGTTTACTTGT	CGGTTTCAAGTATACTTGT	0,0
OMS00114	T	G	GGATGATGCTGTGAGTCGAGAAAG	ACCTTCGCCACCCATGTTTTATT	AAACGTTTCACATGCACC	AAACGTTTCACTGCACC	0,0
OMS00116	T	A	GCCTTTCTCCCATATCACCATTGCA	AAACGCATCTTACACTGTGTTGTG	CTTTTACATTTTCAATATTCTG	TTTACATTTTCAATTTTCTG	0,0
OMS00118	T	G	GCTTATTAGAGTGCATGCCAGATG	TGGAACCAATGGGACAGTCTCTA	GCGGGGTGTGC[AG]CATT	GCGGGGGTGC[AG]CATT	0,0
OMS00119	A	T	AGCGGCAGCTGTGTTAATGAGA	CTTCTAAAGCTGACAGTCTGT	CCACAGCACTGCTGT	CACACAGCACTGCTGT	0,0
OMS00120	A	G	GGCAGAAGAGGAGAGAGATATGATTG	CCTCAAATACCTCTGACATTGAAGGTT	C[GA]CCCACTAAAAC	C[GA]CCCAACAAAAC	0,0
OMS00121	T	C	GGAAGGAGTCCAGTGTGAGT	AAAATATGCAACCACTAAAAGTGAAAA	ACAGCGTGATAAATT	CAGCGTGTGTAATT	0,0
OMS00127	T	G	CACCTTTCTCTCTCTCCATCTCA	AGTGTGCTACACAACCTTAAAAAATATATATCTATT	CACACACCCAATGTA	ACACACCCAATGTA	0,0
OMS00128	T	G	ATGAAAGAACTCCCAGACAGTATTTT	ACATTTTAACACAGTAACACTAATACACACCA	ACTCTCAGAATTAATTATG	CACCTCAGAATTCATTATG	0,0
OMS00129	C	G	GGAGATGATGAAATAAAAAATTGAGGAAAAGATGA	TGCTGGTGAATTATCGCAATAACCA	TTGAACAACAAGAAAA	TTGAACAACAAGAAAA	0,0
OMS00132	A	T	GTTTATGACTCCATTGCCGAATGATT	AGCGAGCTGCAATTCTATCAATA	CAGCAGTCTCTGTGTGG	AGCAGTCTCTGAGTGG	0,0
OMS00133	A	G	GACCACTTCACTCATTCTCCTTTT	TCCGGTTTACACACTTCATGCA	CGCTCCATCTTTGTGGT	CGCTCCATCTCTGTGGT	0,0
OMS00134	A	G	GAAACTGAAATGATCCCCTCGTGT	GCTAGCATAACAGCATTGCCATAT	TCTATAGCTGCAGTATATTA	TAGCTGCAGCATATTA	0,0
OMS00138	T	G	TCGGACCACATGAGCAGTTC	GTTCAACAGGTGCCACAC	CTAACAAATAACCAAGACTG	CTAACAAATAACCAAGACTG	0,0
OMS00143	T	C	GGAGGCACGCCCCAAA	TTTGTTAAATAGAGCCCTTAGTGGGTTT	CCTGATCCAGAATCTAGA	CCTGATCCAGAGTCTAGA	0,0
OMS00149	T	G	GGCATCATTGTTCTTGCTCTGTTTA	CCTGGGAGGGTTTATATCGGAGTAT	GCTAAATGCACAG	GCTAAAGGCACAG	0,0
OMS00151	A	G	CTAACGTCTTCCAATGATATTTTCAACAAGATA	ACCGTGGAAATACAATTTTATGCCAAT	TCATGACCTTGATAATC	ATGACCTCGATAATC	0,0
OMS00153	T	G	ACTTTGCACCATAGGCTTGACAT	TGTAAGGATGATCAAAAAGCTGAAGTATGTA	ACAAAATGTAATTTTCC	CAAAAATGTAATTTTCC	0,0
OMS00154	A	T	GATGTTGGCTGGAGGTGTAGT	TGGGAACACTTTGCCTACCC	ACAGGGCTTCTGATTGA	AGGGCTTCAGATTGA	0,0
OMS00156	A	T	GAGCAGAACACATAGAGGAAAGACT	GTAATCACCTCTTAGCCTGTATGG	TGTGTCTCTGCTGTAACA	TGTGTCTGCTGAGTAACA	0,0
OMS00164	T	G	CAGAGGAGAGGAGAGCAAAATACTT	ACAACCTACTCATTTGAAACTCATTTGGA	CCAGATTCAATTAAATTTA	CAGATTCAATTCAATTTA	0,0
OMS00169	A	G	AGCACTTGACTCAAACACATAAATCA	CTGAGACAGGAAGAACAATGTTAACAAAA	CAAAAAGCATTGATATCAAT	AAAAGCATTGACATCAAT	0,0
OMS00173	T	C	TGGAAGTAGCTACTTAACAGGAAATGG	AACACGTGTGCTGTTTGTGCAA	CATTAGCTTGTGATGAACT	ATTAGCTTGTGTGAACT	0,0
OMS00174	A	C	TGACTAACTATGAGCCTGAAAGG	GGGATCTCTTGTATAAACTGTGTGGTTAGTA	CAAGAACAGG[AC]TAAATGT	CAGAACAGG[AC]TAAATGT	0,0
OMS00175	T	C	TTGCGATATGGGACTGTATACATTTATTCC	ACTACCTCCAGTAAAATAGTGTGGGAAA	ATCACTAGTTCAAATACAA	ATCACTAGTTCAGATACAA	0,0
OMS00176	T	G	GTTGGAAGTCCGGTGGTAGAG	CTGGGTCTGAAGGAGCTT	TTCCAGCACTGCTGTC	CCAGCCCTGCTGTC	0,0
OMS00179	A	C	GTCATAACAAAATCAGGGCTTTCCAA	TGGGAGATTTGGGCTGCTTAAA	TGCCTCTTCTTTTTCTCAT	CCTCTTCTTGTGCTCAT	0,0
OMS00180	T	G	GCGCCGAATGGCATTAGG	CACATTGCTGTGTTTAGTTGACT	CTAAAAGTGCAATTAAGCC	CTAAAAGTGCCCTAAGCC	0,0
Omy_1004	A	T	GAGAATCGGAGCTAATCTTAGTTATTGTGA	CACTTTATTGAGCTACATGGCAAACTCG	CATGTGATGTTTTTTTG	ATGTGATGATTTTTTG	0,0
Omy_101554-306	T	C	GCCTGTATTTCTCCTGTATGTGAT	TCAACTTTTGCAACTTTTTTATTGTCATTT	TGCTTCTCAGCTTTTTTA	TGCTTCTCAGCTTTTTTA	0,0
Omy_101832-195	A	C	TGGCTCTGGACCTGTTGAGA	CGTCACAGCTATTTTAGCGTAGT	TGTAGTCTTTCAGAGTAGTATG	TAGTCTTTCAGAGGAGTATG	0,0
Omy_101993-189	A	T	ACAAAACACAGTGAATTACAATTAACGTT	GGAAGTTAAATTTGCTTCGTGAGAA	CTTGATTTGCAGCTTGTCAA	TGATTTGCAGCATGTCAA	0,0
Omy_102505-102	A	G	CTGCAAACTGACATGGTAGCAAAA	TGCTTGCTTTTTAAAAACAATCTCCA	AACAGGATGTTTTTGC	CAGGATGCTTTTGC	0,0
Omy_102867-443	T	G	CATTTGTTTAAATTTGATTTGGCACAACCTCA	CCCTAGTTCTGTAACACAAGACGTAA	TTTGGGTACATAATTTTT	TGGGTACATATTTTTT	0,0
Omy_103705-558	T	C	CTCCAATCGCAAAATCCAGACT	CGCAGGAGACGGATGCC	AGACTTACCCAGAGTGAGAG	ACTTACCCAGGGTGAGAG	0,0
Omy_104519-624	T	C	CGTGTGAGTTTGGGTAAAGAC	TGACGAGTCCGCTTATCATCCT	CAGCAGGATACATCCGACT	AGCAGGATACGTCCGACT	0,0
Omy_104569-114	A	C	CCGAGCCGACGTGATC	GGCCTCGCTCATCATCA	CGCCACTCCGACGCC	CCAGCCGACGCC	0,0
Omy_105075-162	T	G	GGAGAAGGACAAGGACATTGGTAAT	AAAGCAGACCACACCATACTTCTC	CTTCTCTCTACTTTCC	CTTCTCTCTCTTTCC	0,0
Omy_105105-448	C	T	CAATTTGCAAGCAGGAAAGGTTAT	GTGATGGGCTGCAATTGCTT	AAGGAGAATGCATAATC	TGAAAGGAGAATACATAATC	0,0
Omy_105385-406	T	C	GTAACCTACCCTCACCTGAACCTCA	GTCGCTCTTCTGGGCGTATCG	CTTGAACCAATTGCTAC	TTGGAACCGTTGCTAC	0,0
Omy_105714-265	C	T	CCACTCAGTCAAGCATGGA	GCTTTCAATCCTTGGCTCCAATATC	CTGTTGTTTGAGGTTACG	TGTTGTTTGAGATTACG	0,0
Omy_107031-704	C	T	GGCTTTCGGATACTGAGCAACAA	TGAACTCACTGTTGGTATGAGACTAGA	TGACATGATTGCATAGAC	CTGGACATGATTACATAGAC	0,0
Omy_107285-69	C	G	GCCCTTGTGACAATGCACTGTTATA	AGGTCTAGACAGTGTGCCATTTG	ATACGTACTTTTGACCTTGT	ACGTTACTTTTCACTTGT	0,0
Omy_107336-170	C	G	GCCCTCTCACTCATGACATCAAC	GCTCCAGCCACTCGCA	CACCTCTGGGTGCAGAA	ACTCCTGCGTGCAGAA	0,0
Omy_107806-34	C	T	TCTTTGTCCATGCACATTGATATT	AGCACATTTAGTTAGCAGTGATGGA	ATTGGATGTCAGTGTCATT	ATTGGATGTCAGTGTCATT	0,0

Omy_108007-193	A	G	GTGAATACCACCCAGGCTTGT	GTCCCTTCCCCAGTTTCACTTAATT	ATGTTTTCTCCCTACTTAAC	TTTTCTCCCCACTTAAC	0,0
Omy_109243-222	A	C	ATGTGCACCTCTTAAATTGTAAGTAAATGT	ACCCTATATTCAGTGGCAAGATTGC	TGTTCATTAAATGACTTTTT	TTCATTAAATGGACTTTTT	0,0
Omy_109525-403	A	G	CCTCATTCTCATTGGTGAGTTGTCT	TGTAAGATCTGACCACATGAGTATAACCA	CCTACACCTCTTTTTTCCACA	CCTACACCTCTTTTCTCCACA	0,0
Omy_109894-185	T	C	GGGAGGAATTGGGAATGACAGATTAAC	CGGTGTCATTATGGTTGTCAATTGTG	CTCCCTGATCCCCC	CTCCCTGGTCCCCC	0,0
Omy_110064-419	T	G	GTGCAAGGGACCTAGCTAATCC	TCTGAACTGACACTGAAGAACAAAGAA	ACGTTAGCTTTTAAATTC	AACGTTAGCTTTTCAATTC	0,0
Omy_110201-359	T	G	GGTAAGGCCTGTCTGACTATTTTGA	AGAGGTCAATGGATGCCAGTTT	TTTGGCTATTGAAATTATACATT	TTGGCTATTGAAATCTACATT	0,0
Omy_110362-585	G	A	GCAGCCAAGATGAACGAAAAC TTC	CCGGCCTGGGTCTCAATG	CACCGCCTGCCCGT	CACCGCCTGCCCGT	0,0
Omy_110689-148	A	C	GTGTGTGGCAGAGAACTAACTGAT	GGTTAAGACATTAAACATAACACTGGACTCT	CAAATGAACACATTATTTATC	ATGAACACATGATTTATC	0,0
Omy_111084-526	A	C	CACCACACCAAGCAACTATTTTCATT	ACCCAAC TACTGTCCATTTTTTCAT	CCAGTGAAATTTATTTTT	CAGTGAAATGTATTTTT	0,0
Omy_111383-51	C	T	CACGCGCAATCTCTCGTTTTAC	TCTTTAGGCAACAAGCGTGTCA	AGCAAGCGCACT[AG]GGT	AGCAAGTGCACT[AG]GGT	0,0
Omy_111666-301	T	A	GGGTGAAAAAGAGTGGGACATTTTACA	GTCAATTTCAAGGCCACGACAAT	AGTATAACACAGTAAGACAAT	AGTATAACACAGTTAGACAAT	0,0
Omy_112301-202	T	G	GTAAACCCCTGCCACATAATTAGGT	CTGAGACACTGCTCCAAGGT	AATGCGAAGACAAACT	AATGCGAAGCCAAACT	0,0
Omy_112820-82	G	A	CCTTTCCTTTTGCATTTCTCTACTATTTATT	AAATGAACTCACGTTGACCTCTGA	CGCCGCCAAGTTA	CGCCGCTAAGTTA	0,0
Omy_113490-159	C	T	CATAGTACATTTACAGATAATGTTTTAAAGTGCATGT	CGAGATACCAAAATGCCACAGTTACAT	CATCTGTTTTGGTTTAGC	CATCTGTTTTAGTTTAGC	0,0
Omy_114315-438	T	G	CCTCACC GATCTAGTCAACTTCATC	AGGAGGCTGAGGGAGATTCTAG	TTATGGGCTTAAGGGTC	TTATGGGCTTACGGGTC	0,0
Omy_114587-480	T	G	CAGATTACGTTATTACGTTTGGGAAATTTTTAAGT	GTGAAAGAGTGGGAAATATAATTATAAGGTCAGA	CCTGTCCAAAATTGT	CCTGTCCACAATTGT	0,0
Omy_114976-223	T	G	GACAAACAGCACTTCATTGCAGTAA	GTTGCTCCAGCACCAGGT	ACCGATGGAACAATC	CCGATGGCACAATC	0,0
Omy_116733-349	C	T	GAAATGGACATGCTCACAATTGCT	GATGTGATCAGTTTAGGCAAGGC	AGAGAATCTGATAGTATTTT	AGAGAATCTGATAATATTTT	0,0
Omy_116938-264	A	G	GTTCAATTCATGTTGAAGTGCGACAT	CTCTGCATGCTCCATCCT	CCTGTCTCAATTTTTCTCT	CTTGTCTCAATTTCTCCTCT	0,0
Omy_117286-374	A	T	TGATGTGTTGTTCTCATGGCTTA	CTGTGCATTATTCTGTGATGCTAGG	CTTTCCTCATCACTCTATGG	TCCTCATCATACACTATGG	0,0
Omy_117370-400	A	G	TGCAAAACAGAGGAAAGGGGATT	GGCTTATTTGTTCCGTACTTGCAAT	CAACTCCAATGAATTAA	AACTCCAACGAATTAA	0,0
Omy_117540-259	T	G	GGCAGGTTAAACACAGTCATCTACTATAAA	CAGCATGTTGCTTTAATCCTTCACA	TGTCACTTCAAAGTTTG	TGTCACTTCAACGTTTG	0,0
Omy_117815-81	C	T	CTGCTTTATGCACACCACATTGT	GCTCTTTCTGGAGAACAAGGTACTG	CTATACGGAGACCAGC	CTATACGGAAACCAGC	0,0
Omy_118175-396	T	A	AGGCTTCACACACACATGCA	GACGCGCAACCTCTAGATTATACTT	CTCTTGACAGATACCCGTA	CTCTTGACAGATTTCCCGTA	0,0
Omy_118205-116	A	G	CTGCGGTGGGCTACACA	GCGAGCTGGGCTAGAG	CTACTGAGGCTGAGTGCT	TACTGAGGCGAGTGCT	0,0
Omy_118654-91	A	G	CAGCGTAGACCGTTTCTCATTAT	GCGCCGATGAGCAGCTT	TCAGCTTGCTTGCCGC	CAGCTTGCTGCCGC	0,0
Omy_120255-332	A	T	GCTAGCTAACATTGAAGGGTGAAT	GGCTACAGGGACTTTACAATGGG	ACTATGCCATGAAGTTA	ACTATGCCAAGAAGTTA	0,0
Omy_128693-455	T	C	GCCTGCAGGAGAAAGTAGAGTTA	GAAATGGAAATGGACCCCAATCCT	CACTCAACTGATACCC	CTCAGCTGATACCC	0,1.8
Omy_128923-433	T	C	CTATGTCCTTGGGCAGAACTTACA	ACGTTTCTTTGGGCTGAGACTTATT	CTTCATTTTCATTCACTGTTTT	CATTTTCATTGCTGTTTT	0,0
Omy_128996-481	T	G	CTCATCCACACTGTACAGTACAAGT	CATGCCTTCGTCTCACTAATAACAC	CAAACTCAACCAC	CAAAACCGCAACCAC	0,0
Omy_129870-756	C	T	TCGTTATTTTGCCTCGCGTA	TCCATTGAAGATGTATACATGTTTTGTGA	ACAGGTATTTTCGTGAAATG	CAGGTATTTTCATGAAATG	0,0
Omy_130524-160	C	G	CGAAGGTAGCGATTGGTCGTT	TGCTGTTCTGCTGTGTCTT	ATGGCTTGATCCTCA	ATGGCTTCATCCTCA	0,0
Omy_131460-646	C	T	GTGAAAAGGAATGGAGGAGTACAGT	TGCTAGGACAGGAAGATCATTGTG	AATAAAGCAGAATTTGTACTG	AAAGCAGAATTTATTACTG	0,0
Omy_187760-385	A	T	CGGCTATTCTCGGTAAGAGCT	AAATGCAACCAGAAACGGAATGTC	TCCTTATCCAAAATTATTGTGC	CTTATCCAAAATAATTGTGC	0,0
Omy_96222-125	T	C	GTAAGGAACTAATTGCGGCAACATT	CAGTTTGTCTAACACCCAGGCATAT	AACTACAAC TTAGCTAATT	CAACTGTGGCTAATT	0,0
Omy_97077-73	T	A	GTGTAAACAAAATGACTCTGGGATTCTAG	AGAAGTGGCAATGGTGTGAAGTAT	TGGTGAACATAGAAATA	CATGGTGAACATAGTAATA	0,0
Omy_97660-230	C	G	TCAGTTATGTGTAATCTCATTACCTCTCCAA	AACAGAAAAGGTCTCAATGTATTTTTGCA	ACGTAAC TGTAGCGTTTT	ACGTAAC TGTACCGTTTT	0,0
Omy_97865-196	A	G	TCAGACTTCTGGTTTGTCCATT	CAGCCCTTATATTCACAATTAAAGTGT	ATTAATTAAACAAGCTC	ATTAATTAAACAAGCTC	0,0
Omy_97954-618	C	T	GCTCTGCTTCTCGGCAATA	CACAATTGGTTTTGCACAAAAGTAAAGTATT	CAACGCTTACCGGTGTGT	CAACGCTTACCAGGTGT	0,0
Omy_98683-165	A	C	GCCATTGCCAGAGAATTGGTTAA	AACACAGCACCATCTTAAAGC	AGCCAGATACATATTTGT	CCAGATACAGATTGT	0,0
Omy_99300-202	T	A	CAGTTTGACCCGATGGTGTGA	GATTATGGCGTGCCTTTTGG	TCAGGCATGAGAGAAA	ATCAGGCATGTGAGAAA	0,0
Omy_ada10-71	C	T	TCTTTGAGCGACAAGTCCTTGT	ACCCACACATGAACGCAAAAG	CTTCTGCGTCCAA	CTTCTGCATCCAA	0,0
Omy_aldB-165	C	G	GGGTTAGGTGGATTTGAAGGAGTAA	AGGAAGGTGATGCTGAGAGA	CTAAAATGAAC T[CT]CCACC	CTAAAATGAAC TCGCCACC	0,0
Omy_anp-17	C	A	GGTAATGCCACATGCGGTAAT	CGCTAAATCTGAAAATGTGCTGTTA	CTCTAGTTGATAGTAACC	CTCATTGGTATATTAACC	0,0
Omy_aromat-280	T	C	CTCCATTGATTATGCCGAACATT	GGAGAGGTCAAAATAGCCTGGTA	TCTTGCAAACTCC	TCTTGCAAACTCC	0,0
Omy_arp-630	G	A	CTGCACAAC TTGTTCTGCTATT	ACCAAGTGTCCCTGTAAGCC	CCGCTCCGTCTGCT	CCGCTCTGTCTGCT	0,0
Omy_aspAT-123	T	C	GCCCATTTCACTGATGCTGTGA	AGGAGACCACTCCAAGAGAACT	CCTTCTAGGCAGTCAG	TTCTGGGCAGTCAG	0,0
Omy_b1-266	G	T	TCATGTGAAC TTTAATTGACTAGGAAGTCG	GATATGAAAATATCTGAAGAGTATATTTGGGAAATTGAC	TCTATAAACACATTTTTTC	TCTATAAACAAAAATTTTTC	0,0
Omy_b9-164	T	-	GCACAGAACACAGCCAATATTAACA	GCCTTGACTCTCCCTTCATGAC	CCTACAAC TTAGCTAACGTG	CCTACAAC TTAGCTACGTG	0,0
Omy_BAC-B4-324	G	T	CGTACTTTTCTTTTACAAAATTAAAGTGGAGGAT	GCCTAATATTGGCCTAATGTCCCTCA	CATTGCCAAATACG	TACATTGACAAATACG	0,0
Omy_BAC-F5.284	C	T	CCTCATTTACTGTAGGACCTTGA	ACAACGCCAACAACTTTCTCTTG	CAGTAGGGCGGCAAG	ACAGTAGGACGGCAAG	0,0
Omy_BAMBI2.312	G	T	CGAGCTCATGTCCGAAACTCAT	TTTGACAGCCTCAACTTCTAGGG	CCGAAAGTTCAACTTT	CCGAAAGTTAAACTTT	1.7,0
Omy_BAMBI4.238	T	C	CATGATGAGGAGGACCAAGATGAG	AGGTGTGGTTCAGGGCAG	CACCGCAATCACCG	ACCGGATCACCG	0,0
Omy_bcAKala-380rd	G	A	TTGCTCTCTTCTGGTGCCTTA	CTTCAGGAGAAAGCGCTACTGT	CATACCCATCTATGTCAG	CATACTCATCCTATGTCAG	0,0
Omy_ca050-64	T	G	GTCATACAGAACTGTTTTGTGTGTCAA	ACCTTGAATTTGGTCTCTAATGCTATTGT	CAGTTTGAAGAATATACTC	CAGTTTGAAGACTATACTC	0,0
Omy_carban1-264	G	A	GCAAAGCCTCATCTTCAATCATTTGT	GCAAAACACAAAGTCAGGAATCACTTA	CATTAATATTGCTAATAACACCAAG	ATTAATATTGCTAATAACACTAAG	0,0
Omy_cd28-130	T	C	CACAACCTCCACAGAGACAGTGA	GAGGACAAAAC TACCGTATGGT	CCTGTTCACTCACCC	CTGTTCTTCACCC	0,2.7
Omy_cd59-206	C	T	CGATTGGCCAGATGTTTCCAT	GCTCCGTTGCATAGGTGACT	CAACAATCGAAGGTAAAT	CAACAATCAAAAGGTAAAT	0,0
Omy_cd59b-112	C	T	TTTGGATAAGATTGTCTTATATGACTAAAATGTCATGT	GCCAACGTCCTAGATATGGTGTAAT	CTAAAAGCCTATAGCAAAC T	CTAAAAGCCTATAACAAACT	0,0
Omy_cin-172	C	T	CGCATGGGACAGGTGTGT	GAGAAAGCCTGTAGAACCATGTCT	CGCTCACCGTGGTTAC	CGCTCACCATGGTTAC	0,0
Omy_colla1-525	C	T	CCTCGGCGTGACAACCT	CCCAGAGAATTGGTGCGATTAGG	CTGTTGGGAGAAGAG	TGTTGGGAAAAGAG	0,0
Omy_cox1-221	T	A	CACTGAACTGTAAGCCATTGTGATT	GCAACATGGGAATGATTCATAAATGCA	CGGTAAGACCATTAAAA	CGGTAAGACCATTAAAA	0,0
Omy_cox2-335	T	G	AGCTGGGCTGTATTTGTCAATACTT	CAGCCCCGCACTGTCT	CTTAAAGACAAAAGCCTTTAT	TTTAAAGACAAAAGCCTTTAT	0,0
Omy_crb-106	G	T	GCTCAAAAAGATTCTGCCAAATTCACA	ATTACAATGAAAGTACTTGAGTGTTATGCAAA	TTGCAATGCGTCTTT	TTGCAATGAGTCTTT	0,0
Omy_CRBF1-1	C	T	AGTTCGGTACGGTAGCCTATTCTA	CGCCCCGGTGAGAGTAATTG	CAGAGTCGCCAAAAT	CCAGAGTCACCAAAAT	0,1
Omy_cyp17-153	C	T	GCCCTCCAAGTTCCAAGTGAAAA	CAGGTCATTGATGAAACGTCAGAAC	ATACCTGAGTGTCA TCG	ATACCTGAGTATCATCG	0,0
Omy_e1-147	G	T	GCACTGACTGTTACCAGGAAAGAG	G TACTGCAGTGTTGAGGCTATATCA	CCATCCTGAATCTGATTAA	CCATCCTGAATATGATTAA	0,0
Omy_ftzf1-217	A	T	ACAGGGATGGGCAACTTTGTT	GGATGACCACG TGACACT	TCATGACGAGT TCTGATTT	TGACGAGTTCAGATTT	0,0
Omy_g1-103	T	C	AGTCGTGACAATGAGAAACAGTGTT	CTTGACAAAAAGAAACGTCCCTTT	CTTTTACAATGAAGATC	CTTTTACAGTGAAGATC	0,0
Omy_g12-82	T	C	GATCAATTCGATCGCTCATGAAACTT	CTTCTCTGTTTCTCATTGTGTCTCA	CAAAC TCTCAGGATTAG	AAACTCTCGGGATTAG	0,0
Omy_G3PD_2.246	C	T	TCATGTATCAATTAAGGCATTGTCTGTCT	GTTAGACACAGTGACCACCTCTTT	AGTAAAGCCATTGTTGAGT	AGTAAAGCCATTATTGAGT	0,0
Omy_G3PD_2-371	C	A	GCAGGTAAAGTACACCATAGAGACA	CTCCCCCTGCTTACCAAAC	AGACATGTGGATTGGCA	CAGACATGTGTATTGGCA	0,0
Omy_gadd45-332	T	C	AGAGAAGACTCACTGCTGTTTGC	AAATCAGTTC CACGCTATGCT	TTGCTCCAAAATGG	TTGCTCCGAAATGG	0,0
Omy_gdh-271	C	T	AGGTCAGTCTACTTACAGTATAAAGCAGT	GTCATGTCAACAGAGTAACATAATAAATCTGC	TCACCCTGAAGTGTAGAC	TCACCCTGAAATGTAGAC	0,0
Omy_GH1P1_2	C	T	TGCAITTTGATGGAACAAACATATTTATAATGTGT	CAAAAACAAGGACAATTTCAAGTGACCTC	AAACTGTTGAACGGTAGTG	AAACTGTTGAACGAGTAGTG	0,0
Omy_gh-475	C	T	AAGTTACCAGAAATTTTGCAAACTCAACT	CCATATTTTGTAGGTTGAGCTTTACCTT	CTGAAACTCATGATATACA	TGAAACTCATGATATACA	0,0
Omy_GHSR-121	T	C	CTGTGTATAAGTTTATACAGTCAGCAGACT	TTCAGAGAGAGAAATGGCAGAAAGG	CCTAATAACCATGATAACAGC	AATAACCATGGTAACAGC	0,0
Omy_gluR-79	C	T	GACTGTCTATAGCTATTCTTCTCAAAC TGT	AGAAACTACCAATTGTGATTAAACAGATAGAAAATACAT	CAAGTATTTTGCGTAGGAAT	CAAGTATTTTGCATAGGAAT	0,0
Omy_GREB1_03	T	C	CACCAGCCTCCACTGAAGAG	AAATTGCTGCCCTATCCC	TTTGGGATAGG	TTCGGGATAGG	0,0
Omy_GREB1_05	T	G	TGGGCAGATATGGAAGAACGG	ACCTTCTAAATGGCCTCTGTGT	CGGTGGCTCTC	CGGTGGCTCGC	0,0
Omy_GREB1_06	G	T	GCATAGAGCCAGTGCAAAAACA	TCATCAAATGGCTACCCGGA	CAAGGTAAA	CAAGTAAA	2.75,0
Omy_GREB1_07	T	C	AGGGCCTTCTCTGACACTA	GTACATCACTGGGGGCGAAC	CTGGTATAG	CCGGTATAG	0,0
Omy_GREB1_09	T	G	CCAGTGGCAACCTCAGGTAG	GACTCCAGTCACCCAAGTCA	TCAATGGAGA	TCAAGGGAGA	0,0
Omy_GREB1_10	T	G	GTTCTTAGTGTAAGAGAGACTGAGA	TCTCAGAAAGAAAAC TATGCCACA	AAGATACAATGT	AAGAGACAATGT	0,0
Omy_gsdf-291	T	C	GCATGGAACCA GTCTCTACAAAAG	ATGGAGTGGAAAA TCACAGCATAT	CATAACCCAGAATTATTA	CATAACCCAGGTTTATTA	0,0
Omy_hsc715-80	C	A	CCGGTCTACCTATAGCTGTTG	AGTCAGTCAATTAGTGGTTTGAAATACTATCA	AACTGTATTTGGGAAAAT	ATAAACTGTATTTGTGAAAAT	0,0
Omy_hsf1b-241	A	-	AGCCCCAACTATCCTAAAGCATTTT	AAATCAATAGCTCAGAGAATAATGAACACCA	CAGTGTTTTGT TTTTGTCAAT	AGTGT TTTTGT TTTTGTCAAT	0,0
Omy_hsf2-146	A	-	CCAACAATTGCAGCCTCATCTTAAAT	GGAGCAGAAAAAGGATTGGACCTT	ATAATCTACTA	ATAATCTAACA	0,0
Omy_hsp47-86	T	A	CACATTAAGCACTCCAGGGGA	TTGCAAAAGGCCAAACAGCAAT	CAGGAGTGTAAATGTTT	ACAGGAGTGTATATGTTT	0,0
Omy_hsp70aPro-329	A	G	TGCGTATTATTGTTTTTCAAGGACTTTCAA	TGAATATTTTCAAATACATGCCAATTTCTTCCAA	ACATTTCCAATATTTCAACTAT	CATTCCAATATTTCAACTAT	0,0
Omy_hsp90BA-193	C	T	GGAATCGATGACGACGAAGTGATC	TTCTCCATGCGTGATGCA	CCTCCGCGCTGC	CCTCCGCACTGC	0,0
Omy_hus1-52	G	A	CTTGCCGGAGGGTAGCT	CCACAAC TTTCTCAAATGAATGGAATGT	CCCATCCCTCTCTCTGG	CCCATCCCTTCTCTCTGG	0,1
Omy_IL17-185	G	A	CCACCACACTCTGCAGCTT	TTGACGGGAATCCGAGACTTC	AAGAATCTCACCTGCCCAT	AAGAATCTCACTTGCCCAT	0,0
Omy_IL-1b_028	T	C	ACTGTCTGGCTAGAGCACATTG	ATCTTCTACCACCGACTGTTTTAA	CTGAGGCAACTTTTGT	TGAGGCAGCTTTTGT	0,0
Omy_IL1b-163	T	G	GGAACACAGGATTAAGCCTACTCT	CCTAAAGGCCTAGGAACTAAACTTCA	CTGAGGTCATAAAAATA	CTGAGGTCATACAAAATA	0,0

Omy_II1b-198	A	T	CAAGCAAAATTGACTCCAGCCATTA	TTTAATCTCGGTGCTGAGCTAGTG	GGGAATGAAGCAACAATA	GGGAATGAAGCTACAATA	0,0
Omy_IL6-320	C	T	CGACTGATCTCCTGCAGACATG	CTTGTTCTCGTTGTTCTTCTCTA	CTATAGGAGAGAGGACAACA	ATAGGAGAGAAGACAACA	0,0
Omy_imp1-55	C	T	CGCTGAGAGGATTGTCAA	TTTTCTTTGTTCAGTCTTCTGTCTCG	CGAGATGATGCGTCTACA	CGAGATGATGCATCTACA	0,0
Omy_inos-97	C	A	GATGGACAGGGTCCTCTTCAC	CCTGTAGATAAAACATGGTACCAGGTC	CCTTCTTGATGGTATCC	TCCTTTCTTGATTGTATCC	0,0
Omy_LDHB-1_i2	C	T	ACGCACACTTATCCTTGACAATGTT	ACTGTGACAACAAATTCGGTGACA	ATGGGCAGTCATTCA	TGGGCAATCATTCA	0,0
Omy_LDHB-2_e5	T	C	TGCTAGGTGAGTCAGAGGTACATATT	GACTGGGAAGGCCACCCATAAG	TTTACCTGTCAAC	CCTGTGCAC	0,0
Omy_LDHB-2_i6	G	T	TCCTCGCCAATACCATACATGTC	AGAGTGAAGCTAACACACACATTTCT	CTGTGTTTTGCTTCCCCA	CTGTGTTTTGATTCCCCA	0,0
Omy_lpl-220	C	G	TGACAATCACTGAGCAACTGAACTC	GTCCAGTCTTGCTTCAACTCATTCT	AGTGACAGTCA	AGTCACAGTCA	0,0
Omy_mapK3-103	A	T	GAAGTCATTACTGGTCAGTGGTCAA	GCACAAAACATGAGGAAAGTTGAGA	AATTATTAAGCCTATTTTTT	ATTATTAAGCCTAATTTTTT	0,0
Omy_mcsf-268	T	C	CCAGCATTCTGTTCCCATTTCC	CTTTAATGTAGATTATATTCTTCTGTAGCCACTATGG	AAATAATAGATAAA[CT]CCT	AAATAACAGATAAA[CT]CCT	0,0
Omy_metA-161	T	G	CGCATGCACCAAGTTGTAAGAAAG	AGTGCCACCAAGCGATAAGAAAA	CAAGTAAGTGGTTATATTCT	CAAGTAAGTGGTTCTATTCT	0,0
Omy_metB-138	T	A	TCTGTCCCTGACGCTATAAAACG	GAAGTATTTTCAGCTTAATTTTCACTGTTGAGTT	TTCGCCAAAGAGAAAT	TTCGCCAAAGTGAAAT	0,0
Omy_MYC_2	T	C	CGGTTGCAGAACTCTCATGTTTG	CAGGCCATGTCTTAACTTGCACTTA	CATAGACTTTTTGACCTTAT	CATAGACTTTTTGGCCTTAT	0,0
Omy_myclarp404-111	T	G	GCTGTGGTGCTCATGGGTAAA	CCAGGGCAGGGTGTCTC	CAAAGCCATACGTGGCC	AAGCCATCCGTGGCC	0,0
Omy_myoD-178	A	C	GGTCAAATATTTCAATTACGATTACACTTAGGC	TGGCAAAGCTGTATTCTTCTAAT	TTTTATGAGATATAATTTCC	TTTTATGAGATATCAITTCC	0,0
Omy_nach-200	A	T	CTCATGAAAAACGGGAGAGCAAAG	CAGCGGCTCTTCAGTAGTCT	AACTGACAGAGTCACAAC	CTGACAGAGACACAAC	0,0
Omy_NaKATPa3-50	A	C	GTTGAGCGTGTATTGGGAAAGAG	TTGCATCGGCTTCTGAAAACC	CACTCTGTTTCTTCTTTT	TCTGTTTCCGTCTTTT	0,0
Omy_ndk-152	A	G	AAGAATTGAGGATAAAAACAAATAATATATAAACATGA	CAAACTACATTCATTAAGTCCAGTTTGT	ACCCACTTTCAAAAC	ACCCACTCTCAAAAC	0,0
Omy_nips-299	T	-	GACAGGATAGGAACGGTTTCTCAAT	ATCAGAAAGTTAATTCAATATGTACACGATCCT	CTGGATTTACATGTAAATAC	CTGGATTTACGTAAATAC	0,0
Omy_nkef-241	C	A	AGTGTCAITGATGTCGGCTATTTT	AAACGAATGCCACCTCAGATGTT	CTTCTGTATCATTTTTG	TCTTCTGTATAATTTTTG	0,0
Omy_ntl-27	G	A	GGTGTGTTACTGTAGTTGTGCCTT	TGTGTAGCTAGTGATCCTGATTGTCT	CAGACAAGAGTACCCCAAGAC	CAGACAAGAGTACTCCAAGAC	0,0
Omy_nxt2-273	C	T	CTTTAGAAAAGCCAAGGTATATTTTAACTACTTCT	CTGTCGCCCTCTAATGGTAAGATAG	AAGGCAC	AAGGCAT	0,0
Omy_Ogo4-212	T	C	TGAAAGGTTTTATGCAGGTTATTTTCT	GTGTGTGTTAAATAAGCATTTGATGA	CATTTGATGAGACATCTT	ATTTGATGAGGCATCTT	0,0
Omy_Omyclmk438-96	A	C	CCCGACTCTACTTCACTACTTTCT	GGCCTAGGACAATAGGACTGAAC	TACGCAAAATTAGGTTTAAA	CGCAAAATTAGGGTAAA	0,0
Omy_OmyP9-180	C	G	CTGGATGTGTAGTATCGGTGGAAAA	CACGTGGGACCTCTGATCTC	CTGTAGTAGTCCCGATTGT	CTGTAGTAGTCCCGATTGT	0,0
Omy_Ots249-227	C	T	CTATCTATCTATCTATCTATCTATCTATCTATCTACTTACTGAGA	CCCCTAGATTAACCTGTCCAGTCT	CCCTCTGAGAACTAC	CCTCTGAAAACTAC	0,0
Omy_oxct-85	A	T	CGTCACTGAAACATTACTGTAACATCCA	CATCATCAGCTGTTGGTTTCTTAA	CATCGCTTATTTATGC	CATCGCTAATTTATGC	0,0
Omy_p53-262	T	A	CCCCAACATCCAGTATACAGTTTCA	CCCAAAATGGCAATTTAATAGGATTCAGA	CAAGTAGTATGGAGCTCTAT	AAGTAGTATGGTCTCTAT	0,0
Omy_pad-196	C	T	CAAACAACCCAGTAGTCTCCAAT	GCTTTTCACCCTTTTGTAAATTAAGCCAAA	AAGACAAAGGTGTAATACC	AAGACAAAGGTATAATACC	0,0
Omy_ppie-232	C	T	CTGTTTTAGATTAGAATGTTTTTGGTCAGGT	CTGAACATAGGCTTTTCATTTCAGACAT	AAATAGCGGAGAAAAAT	AAATAGCAGAGAAAAAT	0,0
Omy_RAD103359-45	C	T	GGAGAAGGATGTGCTCCCTG	ATTTGGAGGTGGAGGGTCCA	CCTGTAACGCACAG	CCTGTAATGCACAG	0,0
Omy_RAD10733-10	A	G	TATAGACCCCTGCCAGTCA	ACAGAGAAACCCCGTCATT	AGGGTGAAGAACTG	AGGGTGAGGAACTG	0,0
Omy_RAD10945-51	T	A	GCAGGCGTTCCATTTGGATG	AGATCGACGAAATCGGTGCA	AATGCGCTTTAACG	AATGCGCATTAACG	0,0
Omy_RAD116-59	T	C	GGAAGAAGTGAGAGCCCTGG	CTGTAGTCCACGATCCGCTC	CCACAATGTCAAC	CCACAACGTCAAC	0,0
Omy_RAD1186-59	A	G	CACAGCCTGGATGTGGTTCT	ACAAGTTCGGGAGTTTCCT	CCAGGACATCCAGG	CCAGGACGTCCAGG	0,0
Omy_RAD12439-64	G	A	GGAACTTTTACATCATGTTGACTG	GCACAGAGAACTCCAGGCAA	CTTCTCCGATGTCA	CTTCTCCAATGTCA	0,0
Omy_RAD12566-14	C	T	GTGGACATTCTGCAGGGAT	TCCCACAAATATTTCATACGCACA	ATGTAAACAAATTG	ATGTAAATAAATTG	0,0
Omy_RAD13034-67	A	C	GAGTGTCTCCAGCCCTCC	TCTCTCCGTTGGCCAGAAAC	ATAAATCACAA	CTAAATCACAA	0,0
Omy_RAD13073-16	G	A	GTGAGGGATCACACCTGCAG	GCACCCATTGTAATGTCCC	AAAGGGGACATTACG	AAAAGGGACATTACG	0,0
Omy_RAD13499-13	T	C	GTTACCTGACGACCAAGGT	GCTGGGGGAGCTTTACATGA	CGCCCTGTCCGCCA	CGCCCTGCCGCCA	0,0
Omy_RAD14033-46	A	G	GCAGGAGATTTATTTGGCCCC	ACCCTGTGATCACATACTGTCT	ATAGAGGAATAGAC	ATAGAGGGATAGAC	0,0
Omy_RAD14269-30	C	T	TGCAGGTTCACTCTACAACAGT	CTGGATTAGGACCTGAGCCG	TAGAGTACGACCCT	TAGAGTATGACCCT	0,0
Omy_RAD14541-72	A	T	ACGCAACGTGACGGTCTTAT	ACAGCCGATGACATGAGACA	CTTATAGAGTTTTA	CTTATAGTTTTTA	0,0
Omy_RAD15709-53	G	A	TGGCAGACTTGGATAACACAGA	TGGTTATATCTACAGTACAGTTCGT	ATGCAAGGCTTAAA	ATGCAAGACTTAAA	0,0
Omy_RAD16104-20	A	G	ATTCCAAAACCTGCAGGGGT	TCAGGATTTGGTAAGGTGGCC	AGGGCAAAG[AT]CAAAGG	AGGGCAAAG[AT]CAAAGG	0,0
Omy_RAD1751-18	T	G	TCCATGTTCTCTGAGGAGTG	TGAATGGACTGGTGAAACCACA	GGAGGTGTACACCT	GGAGGGGTACACCT	0,0
Omy_RAD17632-23	C	T	AAGCTCCTGCAGGTCACTC	TCTGTGAACGTCTTCTGCAAGT	CATGTGAGACCTTTGCA	CATGTGAGATCTTTGCA	0,0
Omy_RAD17849-16	G	C	GACTCCACAGCTACATGGG	CCGTTAATGCCAGGGGAGTC	AGACGGACTCCCC	AGACCGACTCCCC	1,25,0
Omy_RAD18903-48	A	G	GGGGATGAGTTCTTCGGTGG	CCACCAATCCCCGAAGAA	AGGAGACCCA	AGGAGGCACCA	0,0
Omy_RAD1919-22	A	G	CAGGTACAGACACACAGGG	TACACCAACCCACGTTCTG	CAGGGAGGAGG	CAGGGGGAGG	0,0
Omy_RAD19340-24	A	G	GCAGGAGCAGCATATACATG	GGGGTGATTGAGTGACAC	CATGGAATAACATA	CATGGAGATACATA	0,0
Omy_RAD19578-59	A	G	GGTTGGACACCTCCTGGTTA	TCAACCAAGCAACAGATTATAGCT	GGTTAAGAGTATTC	GGTTAAGGGTATTC	0,1,25
Omy_RAD20917-11	T	C	CGTTGTCGTCTCCAATCAGGA	ACCAGCTCGATGCCATTGC	AGGTTGCGAGGTC	AGGTCGCGAGGTC	0,0
Omy_RAD22123-69	T	C	TGGGAAAGCATAGGAGGGGA	TGTGTGCCTGTCTTATAGCCC	CCAAAGATGTCAGA	CCAAAGACGTGAGA	0,0
Omy_RAD2277-7	G	T	CATCTGTCCAACCTGCAGG	ACTGACATGTTGTCCACCCA	TGCAGGTGCTGGCT	TGCAGGTCTTGGCT	0,0
Omy_RAD23354-66	A	C	CTGCTAGTCAAGTCCACCGG	CCTCCAGTAGTAAGGGCTGC	AGCCTGAATGAGGT	AGCCTGACTGAGGT	0,0
Omy_RAD23577-43	T	C	AATAGGAACCAAGCCCGAGC	CAGAGCCTGAACCCATGGAG	TCTGGCTCTGTCGGTCT	TCTGGCTCTGTCGGTCT	0,0
Omy_RAD23894-58	A	T	TGCAGAAAGGCTGTGTGGAT	TCTTAACACAGTCTCATGGAACA	GTGGATTAGGGG	GTGGATTTGGGG	0,0
Omy_RAD24287-74	A	G	ATTGTCTGTCTGCCGAGGTG	TGGCGACCTGTCACTAATGC	GGTCACTACCTCCC	GGTCACTGCCTCCC	0,0
Omy_RAD24343-29	A	G	TGCAGGCAAGATTGGTAGACT	AGGAATGTGAGTCTTATTCAGTACA	TAGAAATACAAGCA	TAGAAATGCAAGCA	0,0
Omy_RAD25042-68	G	T	GCTGCTGAAACTGGTTTGCA	TCATGCAGATGAGCTTCCCTG	AATTTCTGCCCAAA	AATTTCTTCCCAAA	0,0
Omy_RAD25266-23	G	T	TTTGAGAGTGGTGCCGTGCAG	GGGGACAGCACATGGAGAC	GCAGGGGTAGATACC	GCAGGTGTAGATACC	0,0
Omy_RAD2567-8	A	T	CTGTCTGGATAGCCTTGCCC	TCTATCTTGGGGAATAAGCCC	GCAATGGGCTATTT	GCAATGGGCTATTT	0,0
Omy_RAD25907-57	T	C	TCGTAATCGTCTCCTTCA	CGTCTCCGTAATCTAGCATGTGT	TCCTTCATGCCAGC	TCCTTCACGCCAGC	0,0
Omy_RAD26080-69	G	A	TGTGGGACAGCACACTACC	CCAGGACACCACTGGAGAAG	ATTAGTAGCATCATCGAG	ATTAGTAACATCATCGAG	0,0
Omy_RAD26691-36	A	G	TGCAGGAAACCGTCAATCTACA	CAGGAATTAATTGTATGGCCGGA	TCTCTAACAGAAC	TCTCTAGCAGAAC	0,0
Omy_RAD27740-55	A	T	TCGGCCTGTACTAGTCTCACT	GCCTAAATGGCCACTTTCATCA	TAACTTTAAAAAAA	TAACTTTAAAAAAA	0,0
Omy_RAD28236-38	T	C	GGCACACATCTGTCCCAGTAG	GCACTAAGGTCTAGGAGCACG	ATCTGTCTTCGTGC	ATCTGTCTCTGTGC	0,0
Omy_RAD29352-6	A	G	TGTGAGAAAGCGCCTCAAGA	GCCTTACAGTGGTGTGCTGA	GAGAGTAAATA	GGGAGTAAATA	0,0
Omy_RAD29559-69	G	T	TGGGCATCAGAGGCATCAAG	TCAAGTCATCACTGCTCCGT	CTGGCTTTATATC	CTGGCTTTTATATC	0,0
Omy_RAD29700-18	C	A	AATGGAATTGGCCCCAACCC	TCTCCATTGTGTGAATCATGGT	ACAATTCAAATGATTTA	ACAATTAAAATGATTTA	0,0
Omy_RAD2976-26	G	A	AGGACTGTGATCCTCTCAGCT	AGCTCTGCTGAAACATCAGTCT	CAGCTGGGTTGAGA	CAGCTGGATTGAGA	0,0
Omy_RAD30230-25	C	T	ATGCTTTCCTGCAGGTTGT	CCTGTAAGTGTAAGGGATCTGGG	ACGTGTGCTGTAGC	ACGTGTGTTGTAGC	0,0
Omy_RAD30243-74	C	G	TCTTCTTCTCCTGCCCCAG	ACACTGCTTTAGCCATCCG	GGACCGGCCCTCTA	GGACCGGGCCTCTA	0,0
Omy_RAD30392-17	T	C	CCACTACTCAGACCTGCA	GCTCAAGGACCAACAAAAGCT	CTGAGACTGTGTGT	CTGAGACCGTGTGT	0,0
Omy_RAD30619-61	T	A	CTGCAGGTCAATGGGTGCTA	ACACTGATCACATTTTTTGCACACT	CAGTGTAAA	CAGTGTAAA	0,0
Omy_RAD31079-58	G	T	CGCGCAAATGCAGTGATAG	CGCCATGTATTTTCGGACCC	CGATCAGGGCCCTA	CGATCAGTGCCTTA	0,0
Omy_RAD31408-67	T	C	CAACCTGCAAGGCTACAGAA	TGGAGTGCCAAACAAAAGAAGC	ACAGAATGCAGAAA	ACAGAACGCAGAAA	0,0
Omy_RAD3209-10	A	G	CGGAGGAGTTTGAGCAGTCT	CTTCTACCACCACCTCGCTG	CGGTATCCCTGGC	CGGTGTCCCTGGC	0,0
Omy_RAD32139-58	G	A	GCAGGAAACAGGTACAAAGGA	TGGCTTCTTCTTGTCTGAGC	TCGACATGACCTGA	TCGACATAACCTGA	0,0
Omy_RAD33122-47	G	C	CAGGCTTTGTGGACATGTGC	GTGCTCTATCTTGTCTTTGGC	CCACAGGGTGGTGC	CCACAGGCTGGTGC	0,0
Omy_RAD33798-24	T	C	CAGGAGGGTCAAGTGGAGTG	TTGGGCCCTCTCTTTTGGG	GAGTCTATCAAGAT	GAGTCTACCAAGAT	1,5,0
Omy_RAD35005-13	C	T	TGGTCAAAGTTGAGGGTGGT	TTGGGCTCTGATTAACCACT	CCAACCTCCGACGG	CCAACCTCTCGACGG	0,0
Omy_RAD35149-9	G	A	GAGTCAATAGAGCCCCCTGC	TGGTTAGCAGGAGCAATCTCA	GCGCGCTTATGTC	GCGCACTTATGTC	0,0
Omy_RAD35417-9	G	A	GCACCTGACCACATAGCTGG	ACTCCACACTCCACAAAGCA	TGCAGGACGTGCTTTGT	TGCAGGACATGCTTTGT	0,0
Omy_RAD3651-48	G	T	GAGTACAGTGCAGTGTGGGG	CCTTCTCTTGCCACCATCA	GTTGGGAGAACTTT	GTTGGGATAACTTT	0,0
Omy_RAD366-7	C	A	ACCAAATTAGAGCCTGCAGGA	GGAGAGGCGTTTCCGTGATC	CAGGACTTGCTTTT	CAGGAATTGCTTTT	0,0
Omy_RAD36848-7	G	A	CGAGGACGTTTCATAGGGAGC	TGATAAGTCCACCAAGCTGG	TGCAGGGACACCACCT	TGCAGGAACACCACCT	1,0
Omy_RAD36952-53	C	A	TGTAGTCATTGGGGCTGAG	CTACACAGACCAACGATGA	AGGACATATTCATC	AGGACATATTCATC	0,0
Omy_RAD37492-53	G	T	AGTTAGACGGAGCGTCT	ACTGACAGTAGATTTACCATGCCT	GTGGCTTTGTAGGT	GTGGCTTTTGTAGGT	0,0
Omy_RAD37816-68	A	T	CTCATTCCTGGCCGTCTG	CCACTCACACTGGCTTATGC	GCGGCGTAAAAATG	GCGGCGTAAAAATG	0,0
Omy_RAD38269-10	C	A	AAGCCACACCGTTCAACTGA	TGGCTTATCGCGCTCTAGTG	TGCAGGCGCCGGCCCCG	TGCAGGCGCAGGCCCCG	0,5,0
Omy_RAD38406-19	T	A	CTGCAGGGGTATTAGGAGGC	AATGAGTTGTGGCGGTGAGT	AGGCTTTATATGGCC	AGGCATTATATGGCC	0,0
Omy_RAD39156-33	T	C	GGGTGTGACATGTGTGCAGA	ACTGCTTGTCGCCACCAAG	ACCGTAATGGAGAG	ACCGTAACGGAGAG	0,0
Omy_RAD3926-22	T	C	CGTTCCTGCAGGCTTTTAC	TTGGCACAGAGATACGCAG	TTCACTTTCCCTG	TTCACTTCTCCCTG	0,0

Omy_RAD40132-55	A	C	TGCAGGGCCTGTATTGCT	TCAAAGGACTGGGGAGAGGA	TCTGTGCAGTCCTC	TCTGTGCCGTCCTC	0,0
Omy_RAD40520-48	T	G	TGTTCATCTGATCAGCTGTCAG	ACACGTCGGTCTTCTTCTCC	GTCAGATTGCCGCTG	GTCAGATGGCGCTG	0,0
Omy_RAD40641-58	T	C	GGCAAACCTGGCTTGAGTG	AAGGCTCTGCTTCTGCTTGA	AGTGATATCAAGTG	AGTGATACCAAGTG	0,0
Omy_RAD41594-34	A	G	TGCAGGGTTATAATGTGTTCTTGT	AAATCTCGGGCTGAGGAACG	CAGAGATACGTTCC	CAGAGATGCGTTCC	0,0
Omy_RAD42465-32	G	T	GTGGATCTTGAGCTCCAGGC	TAGACATCGGCCTCACAGA	CCAGGCTGGAAGAA	CCAGGCTTGAAGAA	0,0
Omy_RAD42793-59	T	C	CACGGCTAGTGGCATGTACC	CCACACCTGCATCAGTCTGT	CAGAGAATGCCAACAGA	CAGAGAACGCCAACAGA	0,0
Omy_RAD43117-55	A	G	CGGTCACCAAGTTGAACCTGT	CTCATCACCCGCCAGCTTAA	CCTCTAACACATT	CCTCTGACACATT	0,0
Omy_RAD43573-37	A	G	TGCAGGGAACGATGAAACCA	ACAGACACAGCATTGGCCAA	GAAAGAGAGAGTTT	GAAAGAGGGAGTTT	0,0
Omy_RAD43612-42	T	C	GTGGAGAGGGATTTTGGGGG	TGACAGGACAAACACAAGCCA	AAATGTGTATTGTGTA	AAATGTGATTTGTGTA	0,0
Omy_RAD43694-41	A	C	CCCCTCTCCCTGGCTAGAAT	TCAGGGGGTGTGCTTTTCC	AGGGAAGAGCGGAG	AGGGAAGCGCGGAG	0,0
Omy_RAD45104-18	A	G	TGGTGCTTCAGTGCTGTCAA	AGAGTGAAACTGTGTGCGG	CAAGACACCGCACACAG	CAAGACGCCGCACACAG	0,0
Omy_RAD45246-10	G	A	GCCAAACAACATTGCAGCCT	TGCAGACAGGCATGCATAG	AGGTAGAAATATC	AGGTAGAAAATATC	0,2
Omy_RAD46314-35	A	G	ACTGCATCTTTCCCTGCA	TGAAGATACCCAGAGACACCA	TAGCAATGGT	TAGCGATGGT	0,0
Omy_RAD46452-51	A	G	TGCAGGTAAGACTTGATCTGGA	TGACTCCAACCTAAGTGCATGT	TGAAGTCAGAAGTT	TGAAGTCGGAAGTT	0,0
Omy_RAD46672-27	C	G	TGCAGGAGGTCTTTTCCCTTGT	AACACATTCTTATTGCAATGATGG	GTGGTAGCCCATCA	GTGGTAGCCCATCA	0,0
Omy_RAD47080-54	A	G	TCAAAAACCTGCAGGACTTGGA	TGGTTATATCTACAGTACAGTTCTGT	TGCAAGACTTAAAACGA	TGCAAGGCTTAAAACGA	0,0
Omy_RAD47444-53	C	T	GTCTGTGAGGAGCTGAAG	GGGTGACGTTTTCTTCAGC	GGCGAGCTTGGCCCAA	GGCGAGTTTGGCCCAA	0,0
Omy_RAD47955-51	G	T	AGTGTGCTAGAAATGGGCCTG	ACCATGGGCAGTTCATTTCA	TTGGAATAGAATCTATA	TTGGAATATAATCTATA	0,0
Omy_RAD4848-14	G	T	TGTCCCTCTCTGCACGATG	AGTTGGTAGCTCACTCTCTGT	GAGACAAAGGACAGA	GAGACAAAGGACAGA	0,0
Omy_RAD48799-69	A	G	GCTGAGCCACCTACACACAG	GTCTAACACTCGCAGCAGGT	CATCTAGAATAGAAGT	CATCTGGAATAGAAGT	0,0
Omy_RAD49111-35	T	C	GCAGGCTTAGCATTGCTGAC	GGAACTGGGTGGGAGAATG	TTTCTTATATTTGA	TTTCTTACATTTGA	0,0
Omy_RAD49637-74	C	G	TGGGGAACGGTCATTGATC	ACTCCGTTGTGTGCTAGCTT	CGGCTGGGTCGCGC	CGGCTGGGTCGCGC	0,0
Omy_RAD49827-67	A	G	CCTGTGGTCCAACTCAGAGAC	ATGTTGTGGGCGGAATCTC	TGCTTCAGATGCC	TGCTTCGGATGCC	0,0
Omy_RAD50632-21	C	T	CCTGCAGGCTGGGTCATTAT	GAGCCAGCTGTACCTTCTCC	TCAGCACCTCCAGCC	TCAGTACCTCCAGCC	0,0
Omy_RAD52458-17	C	A	ACGCTGTCCCTGAGGATGGTA	AGCTTAGGCTGGGTCCTG	ATGGCCC[CT]AAGAACCC	ATGGCCC[CT]AAGAACCC	0,0
Omy_RAD52812-28	C	G	AGGAGTCTGTCCCATGTCA	GCTTAAGGCTGTGGTATGTGG	CAACCTG[TC]ATTCCACAT	CAACCTG[TC]ATTCCACAT	0,0
Omy_RAD5374-56	A	C	GCTGTTACCGTGTGATGTTGA	AGAGTTCTGGCCTCTCCCTC	AGAGGGAAGAGAG	AGAGGGACAGAGAG	0,0
Omy_RAD54441-29	T	G	TGCTGAGGAGGAGAGGCG	GCCAGATCCAGCCAATCAGA	AGGCGGGTCTGAG	AGGCGGGGCTGAG	0,0
Omy_RAD55404-54	C	T	GCAGGGTGTCCACTACAGAC	AGGAGTCCTGAGAGTTGGGC	ATTGTTTCTGAAGG	ATTGTTTTGAAGG	0,0
Omy_RAD55997-10	A	C	CATTTTCTACCTGCAGGCTGC	AGCCTACATACATAAAGCCAACA	AGGCTGCAATGTTT	AGGCTGCCATGTTT	0,0
Omy_RAD57916-29	A	C	GCAGGGCCTTAGAAACAGACT	TACAGGCCTCACTGTTCTGC	CAGGGGCCAAACGG	CAGGGGCCAAACGG	0,0
Omy_RAD58213-70	A	T	CCTGATGGGTGCTTCTCTC	AAACGAGCATCATTATCCATAGTGT	TTTTT[TA]AAAAATACT	TTTTT[AT]TAAATATACT	0,0
Omy_RAD58835-15	G	T	GTCTGCTAAGGTCTGCAGG	GCCGACCATGAGAGACCTG	ATAGCTGCTGGGACCCA	ATAGCTTCTGGGACCCA	0,0
Omy_RAD59758-41	T	C	GGCCCCCTTCTTCAGGAAT	CACACACTCAACGGGTCAGT	TGATTGCTACTGAC	TGATTGCCACTGAC	0,0
Omy_RAD59950-44	G	A	GGAGCTCATATCGCCGATGG	GAACCTGTGCACCTGCC	GGAGGGGAAGGG	GAAGGGGAAGGG	0,0
Omy_RAD60135-12	C	G	AGCATACACACCTGCAGGAA	TGGTAGGAGGAGATGCTCTGT	GAACATACCGGAAC	GAACATAGCGGAAC	0,0
Omy_RAD619-59	T	C	CATGGAGAAACAGACCCGCT	TGCTGTGTGTATCTGGGG	TGCTGGATCCCCA	TGCTGGACCCCCA	0,0
Omy_RAD62596-38	A	T	GCAGGACACTGGTTCCCAAA	CCTGAGATTGAGATCACTGGCT	TTAAAAATATATATTA	TTAAAAATATATATTA	0,5,0
Omy_RAD65808-68	T	G	TCCTTCACTCTCGATCGGGA	TCAAAGTGGGCCACTACTGT	ATCGGGATTCACTT	ATCGGGAGTCACTT	0,0
Omy_RAD65959-69	G	A	ACATTTTGGGTTAACAACCCGT	GCTAGCGAAGACCCGGAAGG	TTTTGTCGTTCCCTT	TTTTGTCA TTCCTT	0,0
Omy_RAD66218-58	T	C	CTGTGCAGGGAGACAGCTAG	GTGGTATCTGATGATCGGGG	CTTGAGTGTGTTGGTA	CTTGAGCGTGTGGTA	0,0
Omy_RAD66402-36	T	C	GGTGTGATACCTCAGAGCTCTG	CGTCTCCGGATCGTTCAGAG	AACCACCTTCTCTG	AACCACCTCTCTG	0,1,5
Omy_RAD66834-17	C	T	CTCCTGCAGGTCATCTCTGG	CTGCTTGTGCTCAATGCCTG	TCTGGCTGACACCTTTA	TCTGGTTGACACCTTTA	0,0
Omy_RAD68634-40	A	C	TGCAGGACTCCTTTGAAACGT	TAACGCCAGCTGCATGATGA	CCTCTAACCTGAAT	CCTCTAACCTGAAT	0,0
Omy_RAD69583-33	C	T	GACTCTAGACTGCTCCCTGC	GGCCTCAGTCTCCTTCCAGA	CCGGAGACATT[CT]CGCGT	CCGGAGATATT[CT]CGCGT	0,1
Omy_RAD7016-31	C	A	GCAGGAATATTCAGTGTTGCCA	TCTAAATGTCTGTTGGCGGC	ATAATTTCA TTAA	ATAATTTAATTTAA	0,0
Omy_RAD7210-8	C	A	ACACCACACTCCACAAGCA	GGCCTTGGTCTCCTTCATA	TGCAGGACTTGC TTTGT	TGCAGGAATTGCTTTGT	0,0
Omy_RAD72528-44	A	T	TGATGATCCGGACCCCTCTCT	CCCGATTCCCTCCACAGTT	TTGGAACAAACTGT	TTGGAAC TAACTGT	0,0
Omy_RAD73204-63	G	C	CCTGGGCAATGACCTCCAC	AGCTCCCTCTCTCTCCCTC	GTGCCGCTCTCCACCG	GTGCCCTCTCCACCG	0,0
Omy_RAD7384-50	T	C	GACACGCCCTCAGCCAG	TCGCTAGCCTCCTGCTGTGG	GCCTCCGGCAG	GCCTCCGGCAG	0,0
Omy_RAD739-59	C	G	ACGAGGCTTGTAATGCAGT	GTCCTTTATACCAATGTCTGCTG	GAGTTGGCTATTTT	GAGTTGGGTATTTT	0,0
Omy_RAD73963-73	T	A	CCCTCTCAGGAAAGTGACCAC	GGATCATGTCAATCTGATGAGTTGG	TTTCTTTTGGGA	TTTCTATTGGGA	0,0
Omy_RAD74691-49	A	G	CATCTCAGATCAGCCACCCG	AGCACAAATCTTCTGTTGTGCA	ACTGTGGATTTGCACAA	ACTGTGGGTTTGCACAA	0,3
Omy_RAD76060-20	C	T	TGCAGGGTGT CAGATTGGG	TCCATGGGAAATCCAAATGCT	GGGCGCTGTAGGCAA	GGGTGCTGTAGGCAA	0,0
Omy_RAD76570-62	T	G	GCAGGTAGGTAGGAAGGAAAGC	TCTGACTGGTATTGAAAGGACCA	AGAGGTGTTCTGGT	AGAGGTGGTCTGGT	0,0
Omy_RAD76882-63	A	T	GGTGGGGGCAAACTCAGTA	GTGCTCAGCTTAGAATGACAGATT	CAAAATGAAACTATGTA	CAAAATGAAATCTATGTA	0,0
Omy_RAD77789-54	T	C	AGACAAACCTGCGAGGGGAC	AGCAGGTTAAAAACCAACTGTCA	TAAATTATTTTGACAG	TAAATTACATTTTGACAG	0,0
Omy_RAD78147-27	C	T	GCATTTTAGCCCTCCAAAGTC	CCTTCTTCCAGTTGTTAAACCCA	CAAAGTCCCAGAGA	CAAAGTCTCAGAGA	0,2,75
Omy_RAD78502-57	T	G	GAGAGGCATCCTGTCTAGGG	ACCATGCTCTTCTGTAGGTGT	GGAAATATCACACA	GGAAATAGCACACA	0,0
Omy_RAD78776-10	T	C	CACAGCTTCTGTCAGGGTAA	GCTTGATGTGCTCGCTAGT	GGGTAATCTG GCT	GGGTAAACCTGGCT	0,0
Omy_RAD79314-58	C	T	CACACTGACTCATCCCTCGC	GAGTGCTTTACCGAGCTGCC	AGACCTTGTCT	AGACTTTGTC	0,0
Omy_RAD85131-35	T	C	TTCAATAACTACAGGCAGATGGT	AGTTCCAAATGCACGTGACA	GATGGTATGGTGAG	GATGGTACGGTGAG	0,0
Omy_RAD86706-72	C	T	TTCCCTGTAACGTGCACGC	CCACATCACACCCTGACCTC	TACGTTTTATTTCT	TACGTTTTATTTCT	0,0
Omy_RAD88028-7	G	A	TAGCCCAAGTTCGGTCCAAC	AGTGTCTTTGGTGCCTCTC	TGCAGGGGCTGG	TGCAGGAGCTGG	0,0
Omy_RAD88122-32	G	A	TCAGTGGATGGAGTGCCCT	GGTCTTTGGCCTTGTGCTG	GCTGTGGAGATCAT[CT]CG	GCTGTGGAATCAT[CT]CG	0,0
Omy_RAD9004-13	G	A	TATACCAGCCTTCCCTGGA	CAGAGAGAAATCCCCACCC	TCATCTGAAGGGGG	TCATCTAAAGGGGG	0,0
Omy_RAD92485-64	T	A	CCAGTCAGTCTTGCCCTAGG	GGTACCACAGGATTGGAGG	GTGTAGATATACAT	GTGTAGAAATACAT	0,0
Omy_RAD93580-37	T	G	AGGCAGAGGAGGGTTGTTTG	TGCAGAAGTCAAATCACGAACA	AGTCACCTGGGATT	AGTCACCGGGGATT	0,0
Omy_RAD9408-71	G	T	TGCACATATGGCGGGGAAAT	TGTTGTGTATGAGCTTCCAAC	GCCAGGGGACAGG	GCCAGGGTGACAGG	0,0
Omy_RAD98715-53	T	G	CGTAAACGGGAGCTGATCTG	GCTGGTAAAAATGCTGAGGGG	CAGGACTCTCCCC	CAGGACTCTCCCC	0,1,75
Omy_rapd-167	G	T	CCCAACATGCTCTATTGCAGCTA	AGTTGCATAAGATGAATCAATAAATTAACACAGAT	AAACAATCCCCCCAAA	AAACAATCCCACCCAAA	0,0
Omy_rbm4b-203	-	T	CTGAAATTTGATGAATGGAAGCTGCA	CGTATTCAAGTCGATATACAGTCACGAT	CACGTTATTATGAAAAGGATGT	ACGTTATTATGAAAAGGATGT	0,0
Omy_red1-410	C	T	GTA CTCCCAC TAACATACAGTAGACTCA	GGCACCATTGTGTTTTAGGATGTAG	AAAATATCTGCAAGGAAT	AATATCTGCAAGGAAT	0,0
Omy_sast-264	G	A	GAAGTAGGGTTTGTGACCATGTGA	TGGATTCCATTTTAGGCTGTAATACATCTT	CTAGCCAATGCGTCTAA	ATCTAGCCAATGTGCTAA	0,0
Omy_SECC22b-88	T	C	GGATCCCTCCTTTTAACACAAGACT	CTACAGGATGACTACCTAATTGCTAATAAAAAA	CTGTCTGTCCATATATC	CTGTCTGTCCGATATATC	0,0
Omy_snip09-37	C	T	TAGTTGTATTAACTCTCTTTGAGCTAGA	TCATTCCAGCTCCGTTCTCTTC	TTGTGCTATTGACGCCACAG	TTGTGCTATTGACGCCACAG	0,0
Omy_sSOD-1	T	G	GCCGGACCCCACTTCAA	CAGACTAACCGAACAGCATCAGTGG	CCACAACAAGACCC	CCACAACCAGACCC	0,0
Omy_star-206	A	G	CGTGTGCCAGCCCTTCT	GACCACTGAGATCATTGCTGTGA	TCITTTGGCACTATATCT	TTTGGCACCATATCT	0,0
Omy_stat3-273	G	-	CAGACCTCTCTATCTCCCTATGAG	ACCTCTTTTAAATTGTGCCAAGAA	CCAGTTTG	TCAGTTTG	0,0
Omy_sys1-188	C	A	CTTAAATGGTGCTGGTGTGCTATT	AGTGATATCTTAGTGGGTCGAGGAAA	AAACATGTACGACCTGTC	TGTAACATGTACTACCTGTC	0,0
Omy_tlr3-377	C	T	GTCGCTCCGGGTGCTT	GGCCCAACACTTCCCTTCT	CGTGATTAGGTTCTTC	CGTGATTAGATTCTTC	0,0
Omy_tlr5-205	T	A	GAGCGTATCTGGTATGGTAACAACA	CTCCAGCAGCTTTAGAGAGTTTACA	CAGTAATATTTTCAGTGCCCCG	CAGTAATATTTCTGTGCCCCG	0,0
Omy_txnlp-343	T	C	CCTTCAAAC TAACGCATCATAGACATG	CTCTCACTTGCTAATCCCTTAT	AACTGAAGAGATCTG	AACTGAAGGGATCTG	0,0
Omy_u07-79-166	G	T	CCCCTATATTATTGTATCACCCCTGA	ATTTAAATCCATTTCTAAAAATAAGCAAACCTAACCA	ACTTGGGAATACCCAGCC	CTTGGGAATAACCCAGCC	0,0
Omy_u09-52.284	T	G	TTTGTGTGATTGTTGTGACTTG	TGATGTTATTGCAGGTCTAGCGAAA	ACTGCATTGTTGTAGCTAG	CTGCATTGTTGTGCTAG	0,0
Omy_u09-53.469	T	C	ACAGCCTGAGCGTTTGCA	GGAAACTGGGAGAGATCAAAGGA	TTGCAGCCCTTATTGTG	TTGCAGCCCTTGTGTG	0,0
Omy_u09-54-311	C	T	GTGGCTCCCCAGGAACAAG	AAGTTTCATGTACATTC CAGTTACCT	TGGTAATTATTCAACAGATCAGT	TGGTAATTATTCAACAATCAGT	0,0
Omy_u09-56.119	T	C	CCAAGTGGACCCACCAAG	GCTGAGTTTATAGGTCAGTCATTATACATATTGA	AGTGAGCTGAAACAGACGA	TGAGCTGAAGCAGAGCA	0,0
Omy_u09-61.043	A	T	TAGTCACATCCATAGTAAC TTCC	TGTTGACAGACGAAAACCAATCTCT	CAGTTGGTCTTTTTCA	CTTGGTCCATTTTCA	0,0
Omy_U11_2b-154	T	C	GGGAAGCAGAAAACTGGAAGTT	CCCTGTGGGCTTGATATTCA	AATGATACTTTTCAGATTGTAAC	TGATACTTTTCAGGTTGTAAC	0,0
Omy_UBA3b	A	T	GCCACTCAATGCATGTGTTTCTAG	CAGCTAGCTTAAGTGGGATGCAA	TGGAGATAACGCTAACTATT	AGATAACGCAAACTATT	0,0
Omy_UT16_2-173	C	T	ATTGACTCATTATCACCTTAGTTGTAGCTTCA	GCAGCTACTTGCTGTATCACATGTTTGT	ACAGTCAACAAGGGACTTAA	ACAGTCAATAAGGGACTTAA	0,0
Omy_vamp5-303	A	-	CTGCTTCCCAATTCAGTATCGTCTT	AGGCTGAAGCATTTCTGAGTATGAA	TGGCCGTAGTAGTTGGTCA	TGGCCGTAGTTGGTCA	0,0
Omy_vatf-406	T	C	TTGCTTCATTTTGTCATAACCTTGGG	TGCATGCTCTGACAAATGTTACACT	ATGACTATCCACA	ATGACTGTCCACA	0,0
Omy_zg57-91	C	A	CAC TACACACTCACTCACAAAGGA	AGCAGATAAGCCTTGTGAGTGAATCTT	CACAGACTGCACAGCC	CCACAGACTTCACAGCC	0,0

OMY1011SNP	C	A	AGGCTGGTTGGGATTCACTG	CGCCAAACACTAACTCTGTCT	CTTTACCTCGAAGACAAT	ACTTTACCTCTAAGACAAT	0,0
OmyY1_2SEXY	X	Y	GCGCATTGTATGGTGAAAA	GCCTGGCATATGAGTGTGA		ATGTGTCATATGCCAG	NA

806 Table 2: *Oncorhynchus nerka* GT-seq panel (382 loci). This table contains the primer sequences, in-silico probe sequences for each allele, and correction values for each allele for each SNP locus included in the *O. nerka* GT-
807 seq primer pool. Each forward primer is modified with a 5' ‘small RNA’ sequencing primer site (CGACAGGTTCAGAGTTCTACAGTCCGACGATC) and each reverse primer is modified with a 5' standard paired-end
808 sequencing primer site (GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT).

Assay	A1	A2	Forward primer	Reverse primer	A1-Probe	A2-Probe	Allele Corrections
One_la.11217-58	C	T	AGCTGACAGCCACAAAGTCA	AGGTTAGCAGTGCTTTTAAGGT	ACCTAACCCTAACC	ACCTAACTCTAACC	0,0
One_la.11387-56	C	T	CAGTCACCAAGTGTTCCTCCA	TGACCATGCTCCTTAACATCC	TGGCTTCGGATGT	TGGCTTCTGGATGT	0,0
One_la.11922-46	A	C	CAGGACACCTACAGCACCTG	TGGCTCGAGTGGTTGAAGTC	AAAAGTTAATCAAG	AAAAGTTCATCAAG	0,0
One_la.12158-56	G	T	TCTGACCCACGACGTCTTAT	TTCCAGACGCACACAGTTCA	CTAAAATGAACCTA	CTAAAATTAACCTA	0,0
One_la.12851-49	C	T	CCAGACCGTACAGAATCTGC	ACCAATCACAGGCTGTCTTTG	AATCTGCCCTGCTA	AATCTGCTCTGCTA	0,0
One_la.13961-31	A	T	TGCAGGTTAGTGAAGATAAAATGGGA	TGTCACAGAAACACAGTCTGA	ATTACAAAGAATAT	ATTACACTGAATAT	0,0
One_la.14403-38	C	T	CACCCAGCCTGCCACAAG	ACCAGGCTGTGTCATAACCG	TAGAGCGCGATGAG	TAGAGCGTGATGAG	0,0
One_la.14866-33	C	G	TTGTGTGTGGGTGTCAGGAG	CCGAGCTATGAGGTCAAGAGT	AGGAGGACTTTTGT	AGGAGGAGTTTGT	0,0
One_la.15228-58	A	C	ATACTTTCCTGCAACCCGCC	CTGGGACTTCCATCAGGAGC	GCCTCAGACAATGT	GCCTCAGCCAATGT	0,0
One_la.15933-53	A	G	ATTGGTGACACGGGCAC	GCAAAGGATCTGACAATGGC	GCCCTGCACTGCCC	GCCCTGCGCTGCCC	0,0
One_la.17043-35	A	C	GCAGGTGGAGTTATAGTGCTCA	ACAACCACTACTGAGCTACCA	GTAGTGTATGGTAG	GTAGTGTCTGGTAG	0,0
One_la.20787-45	A	C	TGCAGGGTATGTCATGTTTTGT	GCACAACGTAGCCAAATTGC	AAAAGATAATGTGC	AAAAGATCATGTGC	0,0
One_la.20814-68	A	G	ACATTCAGAAAGGGGAAGGTCC	CTACTTCCCTTCCCATGCC	AAGGTCCATTACAA	AAGGTCCGTTACAA	0,0
One_la.21766-41	A	T	TTTGATCTTGATTATTGTCCAGCC	AAACAGGACGTGCCTCTCTG	TCCAGCCATATGGT	TCCAGCCTTATGGT	0,0
One_la.22268-41	C	T	GAGGGACTGGTGCACTTCAC	TCCCTCATTTTCCCTCCCTC	GGCATCACGAGGGA	GGCATCATGAGGGA	1,5,0
One_la.22351-67	C	T	GGAGGCTATCAGCTTCTGCA	TCCAGTAGTATGTCTTGGAGCT	CAGACATCGCTCAG	CAGACATTGCTCAG	0,0
One_la.23491-46	A	G	GAGGCTTTTCACTTCCAACCC	AGTTATGTTACGGGTCTCATTACCA	TGGCCCAACAGTC	TGGCCCCAGACGT	0,0
One_la.23626-48	A	C	ATGCAAGGGAAGGGAAGTGA	CATCTCTCTCTGTTGTAAAGAAGGG	TGAAAAAACCCCTTC	TGAAAAAACCCCTTC	0,0
One_la.24137-66	G	T	ACTCTCTCTCCCACTCTGCA	TCTTGGATGTCACTGTACTACAACA	AGAGAGAGATGTTG	AGAGAGATATGTTG	0,0
One_la.25746-65	C	T	ATGAATCACATTGGCCATGCA	AACGTTTCAAGTTCGTTGCAG	TGGCTGCTGCAA	TGGCCTGTCTGCAA	0,0
One_la.25904-44	A	C	GCAGGTGCATCTTAAGGGTG	TCACTCAAGAGGTAGAGCTGC	ATAAAAAAATTCA	ATAAAAAACATTTCA	0,0
One_la.27200-35	A	C	TGCAGGCCAGTTATAGATCA	TCTTCATCATCTTTATGCTTTCGTT	TGAGAGTATTTGTA	TGAGAGTCTTTGTA	0,0
One_la.27760-67	C	T	GACCTGCAGTTCCACATCCC	ACCCCTCGCTAATTGGCTC	TGGTGCTCAACGAG	TGGTGCTTAACGAG	0,0
One_la.28530-58	C	T	GCCCGAGGTGATGGACCTTA	TCCACAGACAGCAGTGTGTGT	GCTGGCACTAGC	GCTGGCATACTAGC	0,0
One_la.29135-58	A	G	GCCCCGGGTCTCTTTAATC	TGCAGGATGAAGAGGAGGAA	ATCTGCAATCGTTT	ATCTGCAGTCGTTT	0,0
One_la.30563-32	C	T	TGCAGGCTTATTTCGGGTTT	GCTTAACACGGAAGCCAAGC	TGAACTGCGTTTCC	TGAACTGTGTTTCC	0,0
One_la.32238-60	A	G	CCTGGGATGGCGTGGTTAAA	CTCTACCATAAGCCGCCTCC	CCGGTTGAACGCAC	CCGGTTGGACGCAC	0,1,5
One_la.32514-40	C	G	CAGCCACACCCACCAGAC	CCTGAGCAGCTATGGAGTGG	ACCTTCCCTCCAC	ACCTTCCGCTCCAC	0,0
One_la.35102-40	C	T	GGTTCATGCTTTACAACAGGGG	TAGCACTCCCACCAGAACA	CTTTTACCGTACGA	CTTTTACTGTACGA	0,0
One_la.36888-37	A	G	TGCAGGTTTCACGGGAAGAAA	AGTCAATTGACTCTCGCTGATCA	TTTTTGATATTGA	TTTTTGGAATTGA	0,0
One_la.37709-28	G	T	GCAGGAGAGACGGTGGTTTA	CCCGAGGGATGTGAGTACTG	ACGAGAGGCTCGGC	ACGAGAGTCTCGGC	0,0
One_la.39597-30	G	T	TGCAGGCTCTGGGTTTATCT	ACTAGGTCCTTCTTTCGGGC	TCTTGATGATTGAC	TCTTGATTATTGAC	0,0
One_la.39667-45	A	C	ACCACATGGGAGGAGATGT	AACAAAGATGCCAGGGTCC	TGTAACAAAGATGA	TGTAACACAGATGA	0,0
One_la.40490-31	A	G	TGCAGGCACCATAAGCACTA	AGCCTACTTGCATAGCCTTCTC	TGGATTTAAATGGA	TGGATTTGAATGGA	0,0
One_la.40540-34	A	G	TGCAGGACTCCTAATTCAATTCAGA	AACGGCAGACTGGGTACATG	AGTAGCAATAGCAG	AGTAGCAGTAGCAG	0,0
One_la.40596-58	A	C	ATGAGTACTGTACGTGCCGC	TCCGTTAGTGTGGTTGGACAC	AACCCAAAACCCTG	AACCCAACACCCTG	0,0
One_la.41039-32	A	T	AGGGAAGGGAGCTGGGATTT	CTCTCTTGTCGGGATGTGTGT	AAATAAAAAAATAA	AAATAAAATAATAA	0,0
One_la.41570-62	C	G	TTTGACAGAGAACGACAGGCT	CCTGCATCCTACCAGAGTGC	GGTCACGCCAAGGT	GGTCACGGCAAGGT	1,1,0
One_la.41669-43	C	T	GGAGGTTTTCCCTGTAATGCA	GACTGCCTGCAATGACAAGC	TAATGCACAGCGTT	TAATGCATAGCGTT	0,0
One_la.42159-31	C	T	AAGCATGCAGAGCTCCAGTT	ATGGGGGTGTATCCTGGGTG	AGTTCTCTCTACG	AGTTCTCTTCTACG	1,5,0
One_la.42211-64	A	T	CCAACCACGTCAACTGAGCA	TGCATTAATGTAAAAACCGTTAGCT	ATCTAAGAGGATGT	ATCTAAGTGGATGT	0,0
One_la.43386-37	C	T	TGCAGGTGTCTCAGGGG	TCCCTGTTACACACTTGTGACA	CAAGAAACACATTT	CAAGAAATACATTT	0,0
One_la.44788-39	A	G	TGCAGGTTGGAGATGTGGTG	GCACAAACAGACTGGAACGTG	CAGTGAGATGGATG	CAGTGAATGGATG	0,0
One_la.45935-29	A	C	GCTATATGAGCGACCGGTGG	CCCCAGCTAAATAACAATGAGG	TGGAAGGAGAAGCT	TGGAAGGCGAAGCT	0,0
One_la.48100-30	C	G	AGGATCCTGCAGTTTGTACACA	CCCTCGCTGTATCATGTGCT	CACAATACAAATGG	CACAATAGAAATGG	0,0
One_la.4900-40	A	G	TGCAGGATATTAACTTTAAAGCTGT	TCCATGCTAATTTCTTGCAATTCT	TTTTGCTACCCCAT	TTTTGCTGCCCCAT	0,0
One_la.51402-35	A	G	TGCAGGGGAGAGAACAGAATG	TGGTACACTGTAGGGCTACA	TGTTATCATGATAG	TGTTATCGTGATAG	0,0
One_la.53123-31	A	C	CTAAGACCAGCCACCCGAAC	TCAGTTGTGCAGATCCACAGT	CCCGAACAGCCGAT	CCCGAACCGCCGAT	0,0
One_la.5313-43	A	G	TGGCATGAGGGTTTACTGG	AGGGTGCCATTGGGGATACA	GGGAAGGACCAGGA	GGGAAGGGCCAGGA	0,0
One_la.53797-66	A	T	TGTTACAGACGGTGCCAACT	AGGCATTTTTCTGAAGACGT	CAACTTTAAAAAAA	CAACTTTTAAAAAAA	0,0
One_la.5407-55	A	T	GCAGGAGGGGATAGTTGCC	ACAGAAACACAGCTAGCCCA	GCAAGAGATTATTG	GCAAGAGTTTATTG	0,0
One_la.54542-52	A	G	GCAGGTTGTGATCGTGACCA	TGAAGAGACTACGCCCCCTT	TTGACCAAAAGGGG	TTGACCAGAAGGGG	0,2
One_la.55218-55	C	T	CAGGCCCATACTGAACCGAG	CCTTGGGGTGCGGTTGAA	GGCCCAACTTCAAC	GGCCCAATTTCAAC	0,0
One_lb.56018-52	C	T	AAGCATGACGAGTGTTCG	GCACTCCCTGCCCTATACA	GAAGAAACGTGTGT	GAAGAAATGTGTGT	0,0
One_lb.56276-30	A	G	CTGGCCCTGAGTCGTAGAG	AATAGGCCAGTGTGTGTC	CTAGAGCATGATAA	CTAGAGCGTGATAA	0,0
One_lb.56339-60	C	T	CCGAAACTTTGGAGCGAAC	TCCTTTAACTGTGAGACACCT	GTGGCATCTCATT	GTGGCATCTCATT	0,0
One_lb.56528-40	G	T	GCAGGGTTGTGGCTGTTCTA	CCAAAGATGAAACCAGTTCCTGT	TAGAAATGATGAGG	TAGAAATTATGAGG	0,0
One_lb.56760-39	G	T	TGCAGGTCTGAGAAGAAATCAA	ACTTCATCAACCCAACCCAT	TGTAATGAATATA	TGTAATTAATATA	0,0
One_lb.5780-62	C	G	TTGTGTCTGGCAGTGTTCG	CAGTGGCAGGCACAATGATG	TGTTTCGCCCCCT	TGTTTCGGCCCCCT	0,0
One_lb.5833-45	A	G	CGGCCTTATCGTCAGTCTCC	TGAAGTTGCATGGAAGTCATCA	CATCACTAAGACCA	CATCACTGAGACCA	0,0
One_lb.58946-27	C	G	TGCAGGCCAAACAAGTTATTTCT	ACACACACACTCCAAGCT	TCTGCTGCCTTAGG	TCTGCTGGCTTAGG	0,0
One_lb.58981-44	A	G	CAGCGATTCTCTGCTTTTGA	CCTCTGACGCGCATCAATCT	AGAACTCATTAATC	AGAACTGTTAATC	0,0
One_lb.59232-51	A	G	ATTCTGCGTCCATCAGACC	TCCGGGATAGCCAGAATCA	TGCAGTGATGCCAT	TGCAGTGGTGCCAT	0,0
One_lb.60577-60	A	G	ATCGCACACAGGCCCTCAG	ACATTGAGGTGCGTGTCACT	TGGACGAAGACTGA	TGGACGAGGACTGA	0,0
One_lb.60684-45	G	T	CGGGAGGAGCAAACACAGTA	CATTGACTGACCCCTGCTT	GACGTTTGAGTTAA	GACGTTTATGTTAA	0,0
One_lb.61920-50	C	T	GCAGGAAAGGTGAATTATTTCTGG	TCTGTTCAAGACGTCAATTACCA	TTTTTTTCAAACCG	TTTTTTTTTAAACCG	0,0
One_lb.61967-31	A	G	AACCTAGGACCTTGGCGTTC	GACGGAGGGAAAGCATGGAG	GGCGTTCACGCAAT	GGCGTTCGCGCAAT	0,0
One_lb.63191-29	A	G	GCAGGAAAGTAGCATCTTCGC	GCAAGATAGCCTATCCAGTATACA	TATCCAATGTATA	TATCCAGTGTATA	0,0
One_lb.63942-34	G	T	CAGGATCAGTGGCTGTGTGA	AGTAGCATTGGTCTGGCCC	AGAGGGGGAGTTGC	AGAGGGGTAGTTGC	0,0
One_lb.64367-65	A	C	ATGAGGCTCTGGGATGTGGA	CCTGCAATCTCATTATAACGCA	GTGGACTAATACTT	GTGGACTCATACTT	0,0
One_lb.64886-42	C	T	CGCATGGCAGTGACATACCT	TGCATCTTAAGCCAGGGGAA	ACCTACGCTCACG	ACCTACGTCTCACG	0,0
One_lb.67122-29	A	G	AGGTCAGTCTAGTCCCTCCG	TAGGGTGTGTCTGCACTGAG	CCTCCGGATCATCT	CCTCCGGTCTATCT	0,0
One_lb.68250-26	A	G	TTGCCCTCGTGCTTGGAG	TTAACGGTGATGGAGCTGGC	TGGAGGAAGAAGAG	TGGAGGAGGAAGAG	0,0
One_lb.6939-43	C	T	GGCCTCAGAACCTGTTTGA	TCTGCAATTCTGGTGATGGCT	GTGATGGCGGGGT	GTGATGGTGGGGGT	0,0
One_lb.70246-46	C	T	TTTGTGGAGAGCCTGCGTAG	GTACGAGAAGGCTGGCAGG	GCTATGGCGACTCC	GCTATGGTGACTCC	0,0
One_lb.70297-45	C	G	CAGTGGGCTGTAACAGAGGG	GCAGAGGAAGGGGGTCTTC	GGGCCGGCAACTGG	GGGCCGGGAACTGG	0,0
One_lb.70694-28	C	T	TGCAGGCTTGACTGGAAAGG	ACTCCGGTATCTCCTGTGCT	CAACAAGCTGCTTA	CAACAAGTTGCTTA	0,0
One_lb.70768-40	A	G	GGGCCAAGTTTACCCGTCTA	CCGTAATCGTCCTGGCAGTT	GTCTACTACTGGAG	GTCTACTGCTGGAG	0,0
One_lb.72136-33	C	T	TGCAGGATGATGTCATGGTGT	ACAGTACAGGTTTAGGCTGTAAGA	CCTAAACCTCTACT	CCTAAACTTCTACT	0,0
One_lb.72637-54	A	T	TCCCACTGAACTGGTTGCC	AGTGCACAGAAAACTTTGGGC	AGCGAGAACAAACA	AGCGAGATCAAACA	0,0
One_lb.72787-38	G	T	CTGAGCAGTCTACAGTATCAAC	TGTGTACCATGGGCAATTGT	TTCTGGGGTTTTGT	TTCTGGGTTTTTGT	0,0
One_lb.72992-37	A	G	TGCAGGTTTTAGCAAAGCCA	TCCCATATTTGAGTCGAATTGGT	CAGAAGTACATGAC	CAGAAGTGCATGAC	0,0
One_lb.73420-52	C	T	AGGGTTGCAACATTTTGGGA	AGGGAATCTGGAATCCTCCGA	GAACTTTCGATTAA	GAACTTTGTATTAA	0,0
One_lb.73656-45	C	T	ACTTCCAGGGGGATCTCTGT	TGCATTCTCAAAAGACGGCT	TACAGTACGTATAT	TACAGTATGTATAT	0,0
One_lb.74472-27	C	T	TGCAGGTTTCAAAGCTAGACCA	CAGACCAGACAGAGGGCATG	CACTGCCCTGTGT	CACTGCCTCTGTGT	0,0
One_lb.74818-26	C	G	TGCAGGAAAACAAGAGGACA	TGTAATCTACTGCCCATCGT	AAAACACCAGACGA	AAAACACGAGACGA	0,0
One_lb.75116-33	G	T	TGCAGGGCTGAAATTC AATTCA	TGTGTTGCATGTGAGCCCTCA	CGAAAAGCTTCAT	CGAAAATCTTCAT	0,0
One_lb.75641-68	G	T	TCGGTTGT CAGGTGAAAGGT	CGCTTCTTAAACACCCGCTC	TTCCGGGGAAAGTG	TTCCGGGTAAAGTG	0,0
One_lb.75977-57	A	C	CATCACCGGGGGCAAAC	CTTCTGTGACATCGGGTCC	CAAAC	CAAAC	0,0
One_lb.76686-31	A	C	TGCAGGTGATGTATGTGTGT	CATGGCCTTTGTCAGGGAGT	ATAGTTTAATATTC	ATAGTTTCATATTC	0,0
One_lb.76828-42	A	C	CAGGAGGGAAGGGTGGAGG	AGTTTACTCACCACACCCCG	AGTCACCACCATAG	AGTCACCCCATAG	0,0

One_lb.77679-43	A	C	CCAAGCTGAGCTTCGGAGAT	GAAACCCAGGTAGCCAGTCC	GAGATCCAACGACC	GAGATCCCACGACC	0,0
One_lb.78278-49	C	T	AGCATTCACCAAAGATAGCATGG	CAAAAGCCAAAACGTGCAGC	TACATCACCTAGTA	TACATCATCTAGTA	0,0
One_lb.78365-62	A	C	CGTCACGTGATGTCCCATATA	CTTCAGGTGGCCATCTTGGA	GGCATCCATGTCAA	GGCATCCCTGTCAA	0,0
One_lb.79240-40	A	G	TTGCCCAATGAGGAAAGGCC	CAGCTGGAGAGGGCCATG	TTCTTGCACTCTAG	TTCTTGCGCTCTAG	0,0
One_lb.79575-35	C	T	TTGGCTACACAAAGGACGGT	GACGGGTTGCTAAGGTCAGG	TTGTGGTCGCCTGA	TTGTGGTTGCCTGA	0,0
One_lb.80431-46	C	T	TACTGGCCCCCTAGCTACAG	TGTCTGTCTGTTCCTTGCTTGT	GAAACCACAATGAC	GAAACCATAATGAC	0,0
One_lb.80929-65	A	G	CCAAAGGGGACAGACAGCTT	ATGAGCGGGAGAATGTAGCT	AGACATGAGATTAC	AGACATGGGATTAC	0,0
One_lb.83667-61	A	C	GCAGGATGTACGACACAGA	CGCAGGAGTCCCTAGAGGA	CCAAGTCATCCTGC	CCAAGTCCTCCTGC	0,0
One_lb.85587-38	C	G	GCAGGCAGTCAGGATGTCTC	AGGCAGTTCTAATGACAAGTCCT	AGAGTGTCTTTTTG	AGAGTGTGTTTTTG	0,0
One_lb.88442-61	A	G	AGTAACCAAAAAGTCTCTCGTTCA	GGCTTGCTCAAGTGTTGACA	CGTTCAAATACCCA	CGTTCAAGTACCCA	0,0
One_lb.8859-52	A	G	AGGGACTTGGCTTTCAGCTG	CTCTCTCCCCCTTCTCCCTC	TGGGAGAAGGAGGG	TGGGAGAAGGAGGG	0,0
One_lb.89789-50	A	C	CCAGACGGTCATCTGTGCC	GGACAGGCAGCATGTGTGA	CTGTGCCAATTTGA	CTGTGCCCATTTGA	0,0
One_lb.90162-56	A	C	TCAAGCGACCACCAGATCAC	CCGTGGCTCTTCCCTTTTA	CGCAAGCATGCTAA	CGCAAGCCTGCTAA	0,0
One_lb.90239-52	A	G	CAACCTCTACCACGACCCAC	AGTTGTAAAAGAGGAGAGAAGTGT	CCATCGCACAAACA	CCATCGCGCAAAACA	0,0
One_lb.9134-28	C	T	TGCAGGACGTTGCCTTATCA	AATGGCAAGGTCCCAGTTGG	CTCATTACGCATGG	CTCATTATGCATGG	0,0
One_lb.9362-41	C	T	GCAGGCTCTGGTAGTATTCACA	CGGGGTGCTATTCTCCACTC	AAGGTAGCGTGCAT	AAGGTAGTGTGCAT	0,0
One_lc.34041-34	C	G	GCGTTTGGAAATTGTCTCCA	ACAAGACCTCAGAGTGCTGT	GATGAACCAGACTG	GATGAACGAGACTG	0,0
One_lc.51810-33	C	G	TGCAGGAGGAACCGCCC	GCAAAATGGAGTGTACGGC	CAGAGGACGAGCCG	CAGAGGAGGAGCCG	1,1,0
One_lc.5765-39	G	T	CACAGAGGCTGCTCCAGTAC	TCCTACCTTGACCAACTCCGA	GTACGCCGCCAGCG	GTACGCCCTCAGCG	0,0
One_lc.70294-64	A	T	AACAAAAGGAACCTTGAACAACT	CTTTGTTGCGCCACAGTG	GAATGTAATAAAAA	GAATGTATTAAAAA	0,0
One_lc.85338-32	C	T	TGCAGGCCTATTGTTGATGTCT	TCCTCCTCTTGCACTCCTCT	ACAAAAGCTACTAC	ACAAAAGTTACTAC	0,0
One_lc.87896-59	C	T	GAGGGAGAAGCATCAGAGGT	CCTGGCCCTTTACACCAAGT	AGGTAGTCGCAITTA	AGGTAGTTGCATTA	0,0
One_ld.50813-42	A	G	TGCAGGGTTGGGTAGGTTAC	ACGTTACTGATTTCAATTTTGACA	CTTTACAA.TACTA	CTTTACAG.TACTA	0,0
One_ld.80954-64	A	T	CTGCGGGGACTGATGTCTAC	GGGAGTTACCACACACTGCA	CACTCACACACACA	CACTCACTCACACA	0,0
One_2.10623-54	G	T	GCTAATGTGAAAACGTGCCTGA	TAGCGTGTGTCACAAACCT	GATTCTTGTGTGGC	GATTCTTTTGTGGC	0,0
One_2.11303-51	C	T	CAAGTTTGGGACAGGACAGGA	ATTCTGTAGTGCCCGTTCC	GGATCAACAGTCCA	GGATCAATAGTCCA	0,0
One_2.13049-68	A	C	ACATCGTTGGCAATGGAAGT	TCTCAATCATCGGCATGTGGT	GGAAGTTAATTTTA	GGAAGTTCATTTTA	0,0
One_2.13757-55	C	T	ACAAGGAGTCGCTTGAGCC	GGGAGTCCCATAGAGTTGCG	ACCCTCCCCTAACC	ACCCTCCTTAACC	0,0
One_2.14018-56	A	G	ACCAGCTGTCAGAGATGCAC	GGCATCCTCTCTCTGTCCG	GGTGGTGACCGACA	GGTGGTGGCCGACA	0,0
One_2.15628-38	A	C	CAACTTCCTGTCCAGCGGAT	CAATCTCCAGCTCCCACAGG	CGGATACAGCAGCC	CGGATACCGCAGCC	0,0
One_2.16766-45	A	G	GCCTCGATAAAGCCTGGGTC	TCCGGCAGTTCTCGATTTACA	CTCCCGATGCTTA	CTCCCGGTGCTTA	0,0
One_2.20011-31	A	G	TCCAGCCGGTCACGTTTC	GGGAGAGGCAAAAGGTCAAGT	TTTCGGAAGACGCA	TTTCGGAGACGCA	0,0
One_2.20130-27	A	T	TGCAGGCTTACTCTCTTGGT	GGCATTAAGAGAGGCGAGGT	TGTGCTAAATACAA	TGTGCTATATACAA	0,0
One_2.21362-59	A	C	GCAGGGACACACACACTA	TCTTCCCTTGTGTACTCTTCTTT	ATAGATTACAAAAA	ATAGATTCCAAAAA	0,0
One_2.21498-40	A	C	TCAGAGGTATGGCCAGCCTA	AGCGCACAGATCTGGGAATT	AACATTTAAGCAAG	AACATTTACGAAG	0,0
One_2.21735-50	C	T	TGCAGGAGAGAGGGTCTTTA	TCCTCTGAATCAGTATCTCTCCT	GGAGGCTCGTGTTA	GGAGGCTTGTGTTA	0,0
One_2.21990-53	A	T	GAAGTCGATGTGTTGCAGCA	TGCAAATTTGCGATGAATTGATTT	TACTCTTACCAACA	TACTCTTTCCAACA	0,0
One_2.23230-33	G	T	GGTGTAAATCCAAAAACAGCCCT	TCAGACGATGTGTCCTGATATACAGT	GTTCTCAGGTGCAT	GTTCTCATGTGCAT	0,0
One_2.24434-33	A	C	AGACTCTCTCCCTTCTGCAGT	TCAGTTCTTAAGTCTGTGAGGT	TATAGAGAGAGAGA	TATAGAGCGAGAGA	0,0
One_2.24795-33	C	T	ACAGTCAATCAAGCTGCAGC	GCTCTGGTAAGATGGGGCAG	CAGCTAACTGGGTC	CAGCTAATTGGGTC	0,0
One_2.27413-63	G	T	TCCTTTGAACATCACTTAATTGACA	TCCCTAAAGCAAAGTGACTATGC	TTAATATGGAGAAA	TTAATATTGAGAAA	0,0
One_2.2794-52	A	G	ACGACCTTTCCTCAAGTGGC	GGCCAGCCAACAGAGAGTT	AAGTGGCACAGTTA	AAGTGGCGCAGTTA	0,0
One_2.28376-51	G	T	CTGTGATACCCGATACCCGC	AGAGAAAACAAGAGAGAACTGCC	TGTGCATGGGCAGT	TGTGCATTGGCAGT	0,0
One_2.28955-38	C	T	ATCAGCACTCGCCGTC	CCCATGTATTGGTTTGACGGA	CCGCTCTCGGGCAC	CCGCTCTGGGCAC	0,0
One_2.29037-65	A	G	TCTCCTCTGCAGAGTGTGA	GTCCGGGGAGACACATGAAT	TGTTCTAAGCCCG	TGTTCTGAGCCCG	0,0
One_2.30761-49	G	T	ACCTTGGACAGCTGCTGAAA	CCTGGTGAGGACAACAAGCA	ATTGTGGGTTTTCC	ATTGTGGTTTTTCC	0,0
One_2.32804-64	A	T	TCGGATTTGGGAGTGTGTGG	GGATGGATCGGATGGCATGT	GGATATAATAAACA	GGATATATTAACA	0,0
One_2.34398-57	C	G	GCTCCTTAATCTTGACCCCCA	CTTGCGGTGATAGAGACGG	ACCATCTCGGTCAG	ACCATCTGGGTCAG	0,0
One_2.34943-66	A	C	TGGTTAGATCCTAGGATAATGCAAC	TGTCTTAAACCTGGACCCCA	TTTACATATTTTAA	TTTACATCTTTTAA	0,0
One_2.35396-68	A	G	GTGAGAGTGGAGCTGTAGGC	GGGGAGACTGTGTCGTGTGA	TCATGGAATCACAC	TCATGGAGTCACAC	0,0
One_2.38070-37	A	G	GCAGGGAATGTTGTAGAAACACA	CTGCGAGACTGCTCACAACT	ATATTCCAATAGTT	ATATTCCGATAGTT	0,0
One_2.38869-26	A	G	GGACAGTCTGAGGGTGTTA	TCTCCTCTCCCAGAGCAGAC	TGTTAAGACAAAGG	TGTTAAGGCAAAAGG	0,0
One_2.39254-69	A	T	GGTTCCTTGGAGCTACATCG	TGACAAAAGAGGAGGTGGCTC	AGAACACACTCTCT	AGAACACTCTCTCT	0,0
One_2.39727-26	C	T	AGGTAGTGTGAATAGACGGGGA	CACCACCCATGATCCCAGTG	CGGGGAACATGGTG	CGGGGAATATGGTG	0,0
One_2.40976-37	A	G	CATGTTGTTGTGACGACGGG	CCCCACTTCTTGCCAGTTG	GCCATGTACACTTC	GCCATGTGCACTTC	0,0
One_2.41128-67	C	T	ACTGGCTGCCTTTGTTCCCTT	TCAGTCAGCTGCACAGAGAC	TGAATAGCGTCTCT	TGAATAGTGTCTCT	0,0
One_2.41231-65	C	T	TGCAACTGAGTTTCTTGAGGC	GGATGCGGATGGTATAGCCC	CAAGCTACTGAGGC	CAAGCTATTGAGGC	0,0
One_2.41371-35	G	T	GGATGTTATCCTTAGAGTATCCCTT	ATCATTTTCTGTGGGCCA	CCCTTCAGGTTTCAT	CCCTTCATGTTTCAT	0,0
One_2.44103-35	A	G	GGAGGAAGGAGAGGATGGGA	GGGCTTCTTTCTTGATGTTGA	GAGAGTAAAACATG	GAGAGTAGAAAATG	0,0
One_2.45396-65	A	T	AGTTCAAGGAGAAAAACCCCC	AGTGTTTGAGGACTTCACTGT	CCCCACATGTATA	CCCCACTTGTATA	0,0
One_2.45475-58	A	C	ACAAGGTGTTGTGACGGGT	AGACCTCTGCATCAGGTTG	GCAGGGTAACGCCA	GCAGGGTCACGCCA	0,0
One_2.45776-68	A	G	ATTGTGCAGGTAGACGCCTC	AGAGAATAGGCCTCTCCGCT	CGGAGACATCCTAG	CGGAGACGTCTAG	0,0
One_2.45877-55	A	G	TCCCAATAATCGCCAACCGT	GAGTGAGCCGACAGCAACAAT	AGTATTTATATGTC	AGTATTTGATATGTC	0,0
One_2.45957-54	C	T	CGTTAGTTCGGAATCTTCTTCT	TTTCATCCCGCAGCAGTAGG	GGTATATCGC	GGTATATTGC	0,0
One_2.46012-53	C	G	GTCTCCAGAGGGTTGTGCAT	GCTCAGCTGACAGACAGGAG	CCTACTTCGAACAG	CCTACTTGGAACAG	0,0
One_2.46152-39	G	T	TTTTGTGCTGAGCAAGGTGC	GACCCATGCATCCATCTGCT	GTGCCCTGGTTCCC	GTGCCCTTGTCCC	0,0
One_2.48609-47	A	G	AGTTAGGCAAACACACCGG	AAGGTGGCCAATAGCAAGAC	GGTCTCATGTGAG	GGTCTCTGTGTGAG	0,0
One_2.48637-56	A	C	GCTTTTTGCAGTGCGATGTC	TTGTCAAGATGGGGAGAGGC	TTGATTAAGGACAG	TTGATTACGGACAG	0,0
One_2.48952-59	A	C	AAGAGCACAGACAAGCCTG	CTACAGCCCTAGCATACGGC	GACTGTGAGGCCGT	GACTGTGCTGCCGT	0,0
One_2.51143-68	G	T	TGCAGAAAGGACTTAGGGCTG	GCTCAATCGAAAAATCACCTTCA	AGGGCTGGATTCAA	AGGGCTGTATTCAA	0,0
One_2.51190-49	A	G	AGCACCTAACCCTGTAATACCT	AGGTGTTCTCTAATGTTTTGTACACT	TTCTATCACCTCAA	TTCTATCGCCTCAA	0,0
One_2.52203-44	G	T	GCAGGTACAGTGAGCTCCAG	TGTCCTTGTATCATTACAGAGCCA	CCAATTTGGTTGTT	CCAATTTTGTGTT	0,0
One_2.52394-28	C	G	GAGACAGGCTGCGTCCCATATA	CCCTGGTGCAGAAGATGTGCA	TCCCATACAGCACC	TCCCATAGAGCACC	0,0
One_2.5338-43	G	T	ACCACGTACGTAGCTATGAAGT	ACATTTCTCGAAGCCATTACTGC	GAAGTTAGTGCAGT	GAAGTTATTGCAGT	0,0
One_2.53715-43	G	T	AGCTTCTTTTACAGGCAGCT	TGGTCCATCTCTTAACCCCT	AGCTGGTGGCCAAA	AGCTGGTGGCCAAA	0,0
One_2.54344-58	A	G	CTCCCACTAATGGCGTGAT	AAACAAACTGCATGGCGCTC	TGGACGAATGTATG	TGGACGAGTGTATG	0,2
One_2.5654-67	C	G	GGATGTGCCTGAGACCTACC	AGGGATTGATGATTCATTGATTTGT	GCACTTACTCAAAA	GCACTTAGTCAAAA	0,0
One_2.5744-26	A	C	GGACGGAGGCTGGGATCATA	GTCCCTCATGTGCGTATGTGT	ATCATAAAAAAAAA	ATCATAACAAAAAAAA	0,0
One_2.58200-50	A	T	TCTGTGGGGTATAGAGATACGGT	TAACCCCGCTGTACCAAC	TCAAAAAACATGTT	TCAAAAAATCATGTT	0,0
One_2.58880-45	G	T	GGACAGCCTGGTGTTTGAGA	AGCAGGCTGATGTAGTGCTG	TTGAGACGCTCATC	TTGAGACTCTCATC	0,0
One_2.59897-41	C	T	CGGAGTTTTCCCTGGCACA	GCACCACCCTAGACTGAACA	CCACCGTCAGGGTG	CCACCGTTAGGGTG	0,0
One_2.62240-45	C	G	TGGAGAGGTATTGGTTTCAAGGT	GCTACTAAATAACTTCCATCAGTG	TGTTTGCTCTTTT	TGTTTGCTCTTTT	0,0
One_2.6248-65	A	T	GGGAAAGAGGGAGGAGCCTA	ACTCAAACATGCCTCCTTGC	TCTCATTAGAAGGG	TCTCATTTGAAGGG	0,0
One_2.63402-51	C	G	CCAGAGCCAAAGCCAGGTAG	GATCCCAGACCTGTCAGTGC	CCATTAGCAGTTTG	CCATTAGGAGTTTG	0,0
One_2.63762-51	A	G	TGTGCTGTGTGCTGCTGATA	GGAATATCCCCCTATGCTTTTGT	TCATTTAATGGACT	TCATTTAGTGGACT	0,0
One_2.63984-53	A	G	GTCACAGACATACAGGAGCTCA	TCCACGTAACAACAGGCCTG	AGCTCACAGATCCA	AGCTCACGGATCCA	0,0
One_2.64150-27	A	G	TGCAGGAGAGAGGAGGAATGA	CCACAGGTCTGGAGGTGTA	AATAAAAAACAGTCA	AATAAAAGCAGTCA	0,0
One_2.6529-40	C	G	CAGGGTGCCCTCCACAAC	CCACCCCTGGAGCAGTCAC	TCACCGTCTGTGAC	TCACCGTGTGTGAC	0,0
One_2.65369-59	A	G	GCAGGGAGACACAACAACAAC	AGCTTTGCTTGTGTTGCTC	AATAACAA.GAGCA	AATAACAG.GAGCA	0,0
One_2.65998-48	G	T	CAGGCTCTCACACTCCTTGG	GCATGCACTTTTACGCTGA	CAGAGCCGCTGCGC	CAGAGCCTCTGCGC	0,0
One_2.66245-33	A	C	CAGGATCGTCTGACACCAGC	CCACACACTAAGGAGCCAGG	CGGACAGATGATTA	CGGACAGCTGATTA	0,0
One_2.66468-68	A	G	TGCTTGTGCTCGCATCAA	TGAGGACACTTTATGGATTCTCC	TGTATCCAAACATG	TGTATCCGAACATG	0,0
One_2.68991-62	A	G	AAACTGGTGATTCCTTGGCC	CCCGTTTGGCTTTGGGTAGT	AGTAGCAAAGACCA	AGTAGCAGAGACCA	0,0
One_2.70711-39	C	T	TGCCCTGTTGTGATGAGCAT	GGCTGTGTAGAACGACCCC	AGGTGCTCGTGCGT	AGGTGCTTGTGCGT	0,0
One_2.71025-65	A	C	TATGAATTCCTCCCGCAGCC	TTATGGATGAGCTGGGAGGC	ATTACCCAGTGTGT	ATTACCCCGTGTGT	0,0

One_2.71512-50	C	T	ACCTTTCTCATTTGTGGATTTGAT	CTGCCAAGTCGATGTTCAAACA	TTTGATTCAATTCCA	TTTGATTTATTCCA	0,0
One_2.72182-45	G	T	TCTTGATTAGTGTCCAGCCATGT	TGTGTGCTCTGTCTGGAC	GCCATGTGGTGAAG	GCCATGTTGTGAAG	0,0
One_2.73281-30	A	G	GCAGGTGACAAAGTCTCTACA	CCACTGTACATAAAACCATGCAGA	TCTGTCCACATTCTG	TCTGTCCGCATTCTG	0,0
One_2.73487-56	A	G	CTGTTCCACACCCTCCATC	GTTCGTGTCATGTCCCACCCC	AGCGCACATACTGC	AGCGCACGTACTGC	0,0
One_2.74998-68	A	G	TGCAGGGACACAAGGAGAGA	CCTGCAGTGTCAAAGCACC	ATTGTTGAGGTGCT	ATTGTTGGGGTGTCT	0,0
One_2.75586-34	A	G	TCGTCTAAGACCTGTCAACCA	TGTGTTTTTCCCCACAAAAGTGA	AAATTAAACTGAGG	AAATTAAGCTGAGG	0,0
One_2.75617-47	C	T	GAGAGTACCGGGCAGACAAG	TGGTGGTGCTCTTCCCATAC	ACCTGCTCATGGTC	ACCTGCTTATGGTC	0,0
One_2.75847-69	A	C	GCCCCAGGATCATGTTGTA	AGAAACTATTCTGGGTGCTGCA	TTCCCTAAAACACT	TTCCCTACAACACT	0,0
One_2.76328-30	C	T	TGCACACCATACTCCTGCTG	AAGAACGTCCAGAACACGCT	GCTGAGCCCTGTGA	GCTGAGCTCTGTGA	0,0
One_2.76532-58	C	G	TGCAGAATACCAATTCTACATTCA	CTCTCGCCCCCTTACTGTG	GGAAATGCTTTCAC	GGAAATGGTTTCAC	0,0
One_2.77164-35	A	C	AGGGGGACCATTCCAGACTA	AATGAGGAGAAGCTTGGCCC	AGGCATCAGCCCTG	AGGCATCCGCCCTG	0,0
One_2.78138-52	C	T	CAGGTGGAGGCAGATCACT	CAGGCCCTCAGCTGCTCTAA	ACATGGCCCTGGCC	ACATGGCTCTGGCC	0,0
One_2.78198-36	C	T	TGTACTACACATTCCCTGTCTAG	CGCTCCCAGAACAAACCTCT	CTGTCAGCGTTGAT	CTGTCACTGTTGAT	0,0
One_2.80177-55	C	T	CTTACGGGATCGGTGACCC	ACTTACAACCTCATGCTAATCACA	CGATTGACCAAACG	CGATTGATCAAACG	0,0
One_2.80525-35	A	G	CCTGCCACAAGGGTTGTTA	GTTTGGCCCCCTTTACTTG	TGTTAGAACGCAAT	TGTTAGAGCGCAAT	0,0
One_2.81573-50	C	G	CCTGCACACTGAGGAGAAG	AATAGTTCAGTCTTACATTCTCT	AGAAGAGCAAAGAG	AGAAGAGGAAAGAG	0,0
One_2.84041-67	C	T	CCTGGCCTACTCTGTGTACG	GCTCTGCTGGTCTCTGCTG	CCTGGGCCGACCAC	CCTGGGCTGACCAC	0,0
One_2.8522-64	A	G	ACCAGACCTTCCCGAGATCA	TGAGCTGTGTGACATTATCCACA	ATGTAAGATAATCT	ATGTAAGGTAATCT	0,0
One_2.85340-30	A	G	GCTGAAGGAGCCGTCGG	CTGGCTGACCCCATGACATT	GCTTCACAAGGGAC	GCTTCACGAGGGAC	0,0
One_2.86889-62	A	T	AACACAAGTCCATCTCCAACA	AGACAACACAGGAAGCAGCC	CCAACATATGTGTT	CCAACATTTGTGTT	0,0
One_2.86898-35	A	C	GGCGGTCACTGAGTCTAAGG	TGGTTTTTGGGGTTCATGTTGA	AAAGGAGATCCTTC	AAAGGAGCTCCTTC	0,0
One_2.87240-34	A	C	TGCAGGACAGGGAGCATG	CATCACGTACAGGTCCCCAC	TCCTGGGATTCTGT	TCCTGGGCTTCTGT	0,0
One_2.90371-33	A	C	GCAGGTGCAGAATCATTTAACCT	GGAAGGAGTACAGAAGGTGTCG	GTAACCTAATTTAC	GTAACCTCATTTAC	0,0
One_2.90529-63	A	G	CAGGATCAGAAGGGGAGCA	GTGAGCACACGGTAATTAGCA	AATCAACAATGCTA	AATCAACGATGCTA	0,0
One_2.9840-29	A	T	GGGCTAAGAACAGATTGCGT	AAACAATGTTAACTCGAGGTTTGA	CGTATCAATATCAA	CGTATCATTATCAA	0,0
One_3RFP.12866-45	A	C	GAGCTTTGTGATTGGGGGAGA	TCTGACCAATCGCAGAGCAG	GCTTCTCATGTGTC	GCTTCTCTGCTGC	1,8,0
One_3RFP.18602-33	A	G	TCATCTGTGCATGTGCTATCAC	TTACTCGTGCCAAAGAGCGA	CTATCACAAGCAGC	CTATCAGGAGCAGC	0,0
One_3RFP.19596-43	A	T	AGGCCTTCGACTGCTCTCTA	GATGTTGCTGCTGTGCTTCG	CACTATGACTAGCT	CACTATGTCTAGCT	0,0
One_3RFP.22492-53	A	C	GCAGGAGCCCAAGGTGAG	TCTGTTTTGAGGACATTCAAGTTGT	AATAACTAATCTAC	AATAACTCATCTAC	0,0
One_3RFP.22563-57	A	G	GGATCAAAGCTGGCGAGACA	CCTCAAGGGCCAAGGTTGAT	TTACAGACAAATC	TTACAGGCAAATC	1,1,0
One_3RFP.22594-56	A	G	ATAGAGACCCGACGTTGGCT	CTTGGCCCTAAGCAGAGAGC	GCCGAGCATGAAAT	GCCGAGCGTGAAAT	0,0
One_3RFP.22665-32	A	G	TGACGAGGTTTTAGGAGAGG	GAGTGGAGTGGTTCCAGT	AGTTAGAACCCTCT	AGTTAGAGCCCTCT	0,2
One_3RFP.25184-65	A	G	GCAGGTGCACGCTTTTTAAAG	TCCCTCAGCTGTTTACCAAAC	CATCGCCACTTTGT	CATCGCCGCTTTGT	0,0
One_3RFP.25749-53	A	G	GCCACCACATTAGGGAGGAG	GCCCCATTGCAGGTAATCTC	GCACAGCATTGAGA	GCACAGCGTTGAGA	0,0
One_3RFP.26492-46	A	C	GCAGGACAAGGTACGAGACA	ACCATGTTTTGACATGCTGAGA	ATAACTAAATAATG	ATAACTACATAATG	0,0
One_3RFP.27065-48	A	T	GTCACCTGGACAGTTGATGGA	GCCAATCAGAATGTTTTTCCCCA	ACTGAGGAGTATGT	ACTGAGGTGTATGT	0,0
One_3RFP.28357-29	C	T	CAGGCCTGGGTAGCTCCA	GAGAGAGAGGGGAGGCGATA	GCTCCAAGTGTCTT	GCTCCAATTGTCTT	0,0
One_3RFP.36741-50	A	T	TGTTTACACAGATATGCATGAGA	AAACAATGCTGCCAGTCGCCT	ATAGCCAAGATCCT	ATAGCCATGATCCT	0,0
One_3RFP.38229-53	A	G	GCAGGCCAACCATCAAAG	GCGGTGTGCGTGTGTAC	TAAACACACTCGTG	TAAACACGCTCGTG	0,0
One_3RFP.39193-43	C	T	AGAGTCTGTTTCCCTGAGGG	ACCTTTCTATTTCAGTGTGGAGGA	GAGGATGCTCCTCC	GAGGATGTTCTCTCC	0,0
One_3RFP.40708-40	A	C	TGTTAACACACTTCTTCACACTCC	CCTTCATCCTTGCTGCAGA	ACACTCCAGTGGCT	ACACTCCCGTGGCT	0,0
One_3RFP.43935-63	C	T	CACACCTCCTCCTTAGCCAC	ATGCAATGGCCCAGTGTGAT	AACACATCGGTTAC	AACACATTGGTTAC	0,0
One_3RFP.46202-49	A	C	GGGACGGGTGAGTAAGGTGA	CAGGCAGGAATGTACAAGCG	CGTTAGTAGTTTTT	CGTTAGTCGTTTTT	0,0
One_3RFP.46608-58	G	T	TGGTGTCATGAACACTGAGCT	TTCCGGATTGCTCTCACACC	CCTTCATGAAGTGT	CCTTCATTAGCTGT	0,0
One_3RFP.47148-54	A	G	GCCTTCTCTCAGCGATGACTC	ACCGGTAGCTACACAATTCCC	CATGAATAGAAGGG	CATGAATGGAAGGG	0,1,1
One_3RFP.50303-37	C	T	GCTTCAGCTCTCCCAACAACA	GGCACCTGCAAAGATCTCCA	TCTCTGGCCTGCAA	TCTCTGGTCTGCAA	0,0
One_3RFP.52579-51	A	G	CACCTTGCTCTCTGCCTGTA	TCTGCTCCTCCATGGTAGCT	ATTATTAAC.TAAC	ATTATTAGC.TAAC	0,2,1
One_3RFP.53804-31	A	G	GGTACTTCTTCTCCTGGACGG	GACCTTGGTGTGCGTCTCAA	ACGGATAAGGGGGCA	ACGGATAGGGGGCA	0,0
One_3RFP.53932-47	C	T	TGTTGCCAAGTTGACTAAAGAACT	ACAGTGGATTATCTACCAACAGTAC	AACTAACCACATCAT	AACTAACTCATCAT	0,0
One_3RFP.56125-30	C	T	TGCAGGAGTTAAACAGGAAGTCA	AGCCAAGTGTACTAGTGCCA	CAATTGTCTTATAT	CAATTGTTTATAT	0,0
One_3RFP.58408-56	A	G	TGACGCGTACAAACATCCGT	TGCGTGAATTAGGACCTGCT	AGGAAGCAGAGCAG	AGGAAGCTGAGCAG	0,0
One_3RFP.60219-36	C	T	TGACCTTGGTTGTCAGGTGA	CTTAAGCCGGAAGCCAGCT	ATTGTTTCTCCGA	ATTGTTTCTCCGA	0,0
One_3RFP.60536-30	C	G	CTGAATTTGGCCTGGGGGT	TGTTTTTGCTCAAACCTGGGGG	GTGGTTTCATTTGG	GTGGTTTGATTTGG	0,0
One_3RFP.62278-33	C	G	CACCACCGCTGTCTCAAT	TTCTCCTCCTGTTTCCCCC	CCTCAATCCCATGG	CCTCAATGCCATGG	0,0
One_3RFP.6937-57	A	G	TGGTTTACAGATGCATTGCAAA	ATGGTTTTAAGAGCTGGTTTGG	TTGCAAAATCCAAC	TTGCAAAAGTCCAAC	0,0
One_3RFP.70374-61	A	T	GGGACTGATCATCACGGCTC	TCTTCAGCTTGTGGTGGACC	GCTCATGATTGGCT	GCTCATGTTTGGCT	0,0
One_3RFP.70898-49	C	G	GCACCTTCGGCAATGTGAC	ACCTGAGGAACCTGACCATTC	CTTATATCGTTTTT	CTTATATCGTTTTT	0,0
One_3RFP.70952-46	A	G	GTAAGCTTTGCAGGCCTTCA	TGCGAACCTGGAACATTTGT	TACATGGATGTTAA	TACATGGGTGTTAA	0,0
One_3RFP.71744-45	C	T	AGGCAACCAGAGTTCAGCTC	CACGGCTGCTCTTCTCTC	TGT.TGGCCGGCAG	TGT.TGGTCGGCAG	0,0
One_3RFP.72056-60	A	C	TCAGCCAACGCCTTGATCTT	ACCCAAAAGGACATCTAACCAGA	TTTTAATAATACTG	TTTTAATCAACTG	0,0
One_3RFP.72689-27	A	C	GCCACCCGAAGAAGAACAGT	CCTGTGAATCGTGGGTGAGT	ACAGTGGAGACTAC	ACAGTGGCGACTAC	1,75,0
One_3RFP.74551-60	A	C	ACGGTCTCTCAGTTCCTCT	GCCAAGATATCCAGTTCAGT	AACAACAAAAACAC	AACAACAAAAACAC	0,0
One_3RFP.7464-69	A	G	TGCTGAGTACACACGTCACT	TGGCAGAAATTAACCACTGTTTT	CCACAGGATTTCTC	CCACAGGGTTTCTC	0,0
One_3RFP.76568-44	A	T	GAACTTTCGCCACAGCTC	TTGGGGGCTGGAAACAAACAG	CCTAGTACTTCTC	CCTAGTCTTCTC	0,0
One_3RFP.77278-58	A	T	TTTCCAGCCCCAGAGTTGAG	CCATCACATCGGCACAAAGC	CAACAAAAATGGAA	CAACAAATATGGAA	0,0
One_3RFP.80015-44	A	C	TGCAGGGACAGGGGATTAAA	GTGGTGTCTCTCTGGTCGTG	TAAAAATATAGGAT	TAAAAATCTAGGAT	0,0
One_3RFP.80215-59	A	C	ACTATGCAGCTAAACGCCCT	ACAGCCTGGATGGGATTTCC	TTTCCGGAGTCCAT	TTTCCGGCGTCCAT	0,0
One_3RFP.81817-50	G	T	ATCCGGCCCAAGATGCTTTG	CAACAAGGACAGGTGCCTCT	TGCTTTGGGCACCT	TGCTTTGTGCACCT	0,0
One_3RFP.83488-50	A	C	AGGATTTTAGGGCTTGACCAG	GTGTTCTTTTCTTCTCAGGAAGGC	CTCACTTAATTTTG	CTCACTTCATTTTG	0,0
One_3RFP.89355-44	C	G	TCTATCTTCTCCATGTTCTGGGA	AGTGAGGGAGCATGCAGAAC	TCTGGGACGCGTTC	TCTGGGACGCGTTC	0,2
One_4a.12382-55	A	T	GGTGCAAAGGGAGTCAAATGT	AGTGACCCACACAACCATA	CTACGCCAGAGCAG	CTACGCCAGAGCAG	0,0
One_4a.13104-68	C	G	AACCGAAAAGTGCTCTCCCC	ATCCACTTTCCACAGCTGGA	CTCCCTCTGACAT	CTCCCTCTGACAT	0,0
One_4a.14909-37	A	T	GCAGGACTGTCACAGCTTCT	AATGCAGTCCCAGGTGCG	TTATAACACTTCCT	TTATAACTCTTCCT	0,0
One_4a.19795-33	G	T	TGAATGGTTTGTTACACGCGG	TGCAACAGAGGTGGTTTGGT	CACGCGGGAATCTG	CACGCGGTAATCTG	0,0
One_4a.21799-29	A	G	GTGTGCGTCGCCTGGAG	GCTGTGAAGTAAACCAGCGC	GGTGGGGAAGCTCT	GGTGGGGAGCTCT	0,0
One_4a.22514-58	A	G	CCACTGTAGTGTGCTCTGTGT	GGTGACGCGCTCCACATACAA	TTGTGTTATCTCG	TTGTGTTGTCCTCG	0,0
One_4a.22901-67	A	G	GCTAAGAGCACGATGAGCCA	GGTAATCCCATAGGGCAGCG	CCCCCGACCAAAC	CCCCCGGCCAAAC	0,1,1
One_4a.24016-53	C	T	AGCAGCACACTAGCATGAGC	TTGCAACCAGGAAATGACCA	GTTCTTCAAGATG	GTTCTTTAAGATG	0,0
One_4a.25035-26	G	T	TGCAGGAGCCCATAGGAATG	AGTTCTCCTGCAACATGGTGA	GGCTACAGATGTAC	GGCTACATATGTAC	0,0
One_4a.25174-68	C	T	GCACCTCCCGTGAAGTTGAT	CACCGCTCTAACCCTAGGC	AAGGGGGCGGCAGG	AAGGGGGTGGCAGG	0,0
One_4a.28062-27	A	G	GCAGGAGGAGATCTCTGACC	GCTTTTCCCTCCCTCACCAA	CTTACTGATCAACT	CTTACTGGTCAACT	0,0
One_4a.32159-43	A	G	TGCAGGACTTTTTGCCACAT	GACCACTGATCACTCAGGA	CACCCACAGGCCAA	CACCCACAGGCCAA	0,0
One_4a.32895-28	A	G	TGCAGGAAAAGCTAGACTAGAAT	AGCTTGCCTCCCTTGAATA	ATAGCTTATGGGAC	ATAGCTTGTGGGAC	0,0
One_4a.32932-27	A	C	TGCAGGTTAGTCATTGATAGACT	TCTGTCAAGAGACTAACCTACAGG	GACTGATAGACCTG	GACTGATCGACCTG	0,0
One_4a.40410-31	A	C	TGCAGGACAACAGAGGATGG	AGCCTTCACACTGCACCTTT	ATTCATTAATATTT	ATTCATTCATATTT	0,0
One_4a.41625-38	C	G	TGCAGGCATCAAAGGGGAC	CCCTGATCACTGTGTCTGTGA	CACTTAACACACAA	CACTTAAGACACAA	0,0
One_4a.43537-65	A	T	ACGCAGCACACAGATGGTTA	TGTGTAACCATCTCCTTGCTGT	TTAAAAGACTTAAC	TTAAAAGTCTTAAC	0,0
One_4a.44564-40	G	T	TGCAGGAGTGAATAAGATTCTGT	TCTAGGTAGCCTATGTGTTCAACA	GCTGAGAGGGCAGT	GCTGAGATGGCAGT	0,0
One_4a.46542-38	C	T	GCAGGTGGCAGATAATTGTAGT	CCTATTACCCCTGGTTGGCA	ACATATCCCTCTAC	ACATATCTCTCTAC	0,0
One_4a.47407-42	C	T	AGGAACATGGTGCATCCTGG	ACTGTGTGTGTGTGCGTACA	CCAACAACGCACAC	CCAACAATGCACAC	0,0
One_4a.47733-44	A	C	CTGACCACCTCCTCAAACC	GGTATGTTCAGGGCCCTGTC	ACCTCCAAACTCCT	ACCTCCACACTCCT	0,0
One_4a.5042-49	C	T	GGGGCAAGGGAAAAAGAGGA	TTGCCCGTCCAACATTTCTT	GAAGCATCGGGGAT	GAAGCATTGGGGAT	1,3,0
One_4a.51425-41	C	T	GATGAACACGCGCTGAAC	AGGGTGTCCAGCACAAACATC	GAACGAGCTGCGTG	GAACGAGTTGCGTG	0,0
One_4a.51580-50	G	T	TGCAGGTGCTCTATGAATGTT	CCAGTCTCTTCTGGCTGTG	TCCAAGTGGCACAG	TCCAAGTGCACAG	0,0
One_4a.52596-64	A	G	TGCAGGGAACAAAGGTGTGA	GCCTTACTCGGCCCGAAG	C.TTCAGACTTCGG	C.TTCAGGCTTCGG	0,0

One_4a.53531-43	A	G	TGCACTGCAGAGGAGGGG	AGAGACGATAGCAGCAACATT	CCGTGACACTTATT	CCGTGACGCTTATT	0,0
One_4a.58187-48	A	T	TATCTGGGCTTGGAGGAGAG	AGATGGCTCAGCTGAAGCAA	AGGTCAAAATTCCTG	AGGTCAATTTCTCTG	0,0
One_4a.59326-51	A	C	GCAGGACATAAACTGGGCCT	GCCTGTGTATGCCCTACCC	CAATACAATGGGTA	CAATACACTGGGTA	0,0
One_4a.63494-55	C	G	GCGTCACTGCTTTGTGTTTT	TCCCTCTCCCAGAATGCAGA	TGTCCAGCGTCACA	TGTCCAGGGTCACA	0,0
One_4a.63914-64	G	T	CAC TGTTGAGAGCCGTCACT	TGGGACTAAGTTAAGAGAGTGTGT	GGCTACTGATAAAG	GGCTACTTATAAAG	0,0
One_4a.6566-43	G	T	TGCAGGTGTCTCAGAGGGAT	CCATACCCCCTACCCCTTATCA	TGTAACAGGACAAA	TGTAACATGACAAA	0,0
One_4a.67550-44	C	T	GTGAAGAGCTCTGCCTCCAG	GCCTGAGGTCTCGTACGATG	AGATAGACCTGCAA	AGATAGATCTGCAA	0,0
One_4a.7119-57	A	C	TGCAGGGTAAGTTGAATCAAAA	CCCCAGACCATAAAGCCAC	TCAGAAAAAGTGAC	TCAGAAACAGTGAC	0,0
One_4a.84616-28	A	T	TGCAGGACGGAATACAGAGT	AGGCAGGAGGACATGATACT	AGTATCAAGTCCTC	AGTATCATGTCCTC	0,1,3
One_4a.9496-33	A	C	TGCAGGAGAAGCTTAGCAGG	GCTGAAGAACATGCTTGGCC	GATTGTCTAGAGCTA	GATTGTCCGAGCTA	0,0
One_ACBP-79	G	A	GAGGTGTGGGCTGACCA	TCGACCGCTGGCAGTG	CAGAGGTCAATGGTTCTA	CAGAGGTCAATAGTTCTA	0,0
One_agt-132	A	C	GACCCAGATCAACAACCTTCATCCA	TGGTTGAGTCAAGGTCCTTGAAC	ACAGGAAAAATCACGAGCCT	CAGGAAAAATCCCGAGCCT	0,0
One_aldB-152	A	G	CGATCAGGTGACGCTAAAAATTAAGTCTC	GTGGTCTTCTCTTCACTCTGA	CTCAGGCATTACCTTC	CAGGCATCACCTTC	0,0
One_apoe-83	C	T	CGCCATGGACAAGGTCAAG	GGCACAGTGCTTCCAAACC	TTTAGACGGCGGTCTC	ATTTAGACAGCGGTCTC	0,0
One_c3-98	C	T	GAGTGTGGAAGTGGTTCTTGTG	GCCGGCAGGGCATCA	GTTGATGGACCACCTGGTGG	TTGATGGACCACCTGGTGG	0,1,65
One_CD9-269	C	T	ACGCTCTGAGGTGATATGAAACAC	CATCCGACGTCAACATCCAAAC	TGGAATGGAGAAATC	ATGGAATGAAGAAATC	0,0
One_cetn1-167	A	C	CAGAAATCCTGACTGTTAAAAAATGCA	CTGCTCGTTGATCTCTCCATCTC	TTGACGAAGCAGACCGA	TTGACGAAGCCGACCGA	0,0
One_CFP1	C	T	CGCAGGTCAAAGTAGTACTTAGCAT	GAGCGTCACTTCCTGGAACCTT	TGCAGTTCAACATCAA	CTGCAGTTCAATATCAA	0,0
One_cin-177	C	T	CCTCAGACTAGTGACCGTACCTA	CGCTCACCGTGGTTACGT	TCACGCACGGGACAG	CACGCACGGAACAG	0,0
One_dds-529	A	G	CATAATGCTCCCATCTTGAATTGG	CACTCAGCCCTTTAGGGAAGA	AGCAATCCCATCTCTC	AGCAACCCCATCTCTC	0,0
One_DDX5-86	C	T	CTCCACATTGATCTGGACGTA	TGCCACTTGGCCCAAAGAG	AGGACTTCCTGAAGGAC	AGGACTTCCTAAAGGAC	0,0
One_E2-65	C	T	GTGGCACCCCTTTCTCT	TGCAAAACCTCAGTGGAGAACC	CATTGTCCCTAGGAAAG	ATTGTCCCTAGAAAAAG	0,0
One_gdh-212	C	A	CCTGTGTTGAAGTGGAGTAGGTTAA	GCTTTTACTGTAAAGTGGACTGACCTT	ATCTGTTACCAGAATGTTT	ATCTGTTACCATAATGTTT	0,0
One_GHII-2165	T	A	GGCATCAACCTGCTCATCGA	TGCACAAAGTGCGGCAC	CACAAATGGAAATTGA	CACAAATGGTAATTGA	0,0
One_ghsR-66	A	T	TGTAACAATACAAGGATAATGCAAAATATGTAGGT	GGTTATTAGGTTACTGTGCTGACTGT	AGGTTAAGCTGTGTATAAGT	TTAAGCTGTGTATAAGT	0,0
One_GPDH-201	T	C	GAAGCTGATCCTAGACCTGTACCTA	TGGTATGATGGTGTACTGGAAGT	CTTCACCCCTGGAGCC	CACCCCGGAGCC	0,0
One_GTHa	A	G	CAAGAAGAATCAAGAGAAAGAGAGATGGT	CCTAGTGTATGCACATAACGTGTA	CAAGAACTAGAATGAAACAGA	AAGAACTAGAATGGAACAGA	0,0
One_HGFA-49	A	T	ACTTGCTACTTCAGGGTTTTTGTGA	TGGCAGAACAAATTCCTCAATGCATA	CTAAAGCACCATGTTGC	ACTAAAGCACCTTGTTC	0,0
One_HpaI-99	C	T	CCTGAGTTGTGTTCAATGGGCATAA	TGGGTGATGTTTATTAGAGCACAAA	AACGGAAGAAACCCCTCAA	AACGGAAGAACTCCTCAA	0,0
One_hsc71-220	C	A	ACAGCGAAACTATTGATTTAAGGCTCAT	CGCAGGTAATCACTGATCATGTTT	ATTGGCCACAGCGC	ATTGGCAACAGCGC	2,0
One_Hsp47	A	G	CGTTCAAATAAATGCTGTTTGGCCTTT	GTGGTGTTCGGATTTTTCTGTAAA	TTATTGACTATGGCACATTG	TTGACTATGGCGCATTG	0,0
One_IL8r-362	C	T	TGCTAGAAAGCGTTGGTTATGATGA	CAGCGAAATGTGAGAAGTCACTAGGAAAA	AGCCAAAGAAAGAGTC	AGCCAAAGAAAGAGTC	0,0
One_ins-107	C	T	TTGAAATGAATGTGAAGGCA	GAACCTGCAAGAGGAGAA	TACAGTAGTCCATACAACATA	ATACAGTAGTTCATACAACATAT	0,0
One_KCT1-453	G	T	GGGAAAGTATGCTGTGGGATCAG	GGTTCCTCAGTGAGTGTTCTCTATG	TGGTCAGGGTATCGCCATA	TGGTCAGGGTATCTCCATA	0,0
One_KPNA-422	A	G	TGGGCCCTGGGAAACATC	CCATAGCCACTTTCGATACAGGTAA	CTGGTATGAGAAGGCACA	TGGTATGAGGAGGCACA	0,0
One_LEI-87	A	G	ACAGCGCATCCCCATAATGG	GCCTTGTGGAGGTCAACGA	ACTCGCCACCTCTGT	TCGCCGCCTCTGT	0,0
One_lpp1-44	C	T	GGTCCAATAGGGAGCTCAGACA	GGGAATGAACAGACATGTGAATG	TTGTGCTTTCCTGACCTAT	TTGTGCTTTCCTAACCTAT	0,0
One_MARCKS-241	T	A	CCTATTCACAGCTTGGTTGAGTTCAA	TGACCCGCTCATTTTTTGTAAGAT	TTGCTTAAAGAGTCTTCC	TTGCTTAAAGAGTCTTCC	0,0
One_metA-253	C	G	TTCTTATCGTGGTGGCACTTT	GACCAAAGACTATTTAGTTGCCACCTA	AGGCAATTGAGGTTAAT	AGGCAATTGACGTTAAT	0,0
One_Mkpro-129	A	G	TGACGTATGTGCAATGCATGTCTAT	AGATGAAGGACATGGCTGAAACAT	ATGCATATACATGTAATATAT	TGCATATACATGTAACATAT	0,0
One_ODC1-196	C	T	CCGAGGTGGGATTCAACATGAC	TGTCCTCAGACCCAGGGAAA	CCACCTCCGATGTCC	CACCTCCAATGTCC	0,0
One_Ots208-234	-	A	CAGCCGACATGCATCAGTTA	TGACCCCATGTTTCATGCT	CACACGTTACATCAGATAACT	CACACAATGTTACATCAGATAAC	0,0
One_Ots213-181	T	A	CCATAGTGTATCACACAATACTCATGTCT	TCTATCATCTGCAAACTCTGTGCTACTAGACT	CTTTGAATTAACCAATTTTTT	CTTTGAATTAACCAATTTTTT	0,0
One_p53-534	C	A	GACAATCTTAAAGCGGTGGTCTTG	AACCTTATCAGCCATCATCCAACCT	TCCAAAGATCTGG	TCCAAATATCTGG	0,0
One_pax7-248	C	A	AGTAAAGGTAGTGATGCAATGATGCA	AACCGCATAGGACGTAAAGCA	AATTCAAAACGAAATGTG	TGAATTCAAAACTAAATGTG	0,0
One_PIP_3	C	T	ACAGAGTCAGGACTTGATATGTACAGA	CCTGACGAGGGTCTACTACACT	AACACACATTTCTCAACACA	ACACACATTTTCAACACA	0,0
One_Prl2	G	T	ACCTCTCTCTCTCTCAGGACTCTCA	GAGGAGGTGTGACATAGATGGA	ACCAATGGGACGAGTG	CCACCAATTGGACGAG	0,0
One_psme2-354	A	G	TGGTCCTTCAGGTACTTTTCAGAGA	CAAAATGCCAATTCTCACCACATGA	TGATGCAGTAGCTAAAG	ATGCAGTGGCTAAAG	0,1,8
One_rab1a-76	G	T	TCGCCATATTTCTCTCCCTATCC	ATCCACTCAGACCCATATCTACCAA	TGTGGAGCAAGGTAAC	TGTGGAGCAATGTAAC	0,0
One_RAG3-93	C	T	AGATAAAGATGGTTTCAAAGTACCCCA	GGGCTGCCATCTAAAAATATTGCT	CATTTTGGACTTCGGGACC	CATTTTGGACTTTGGGACC	0,0
One_redd1-414rd	A	G	GTGGCTACATCCTAAACACAAATGG	CAGCCCTGGAGTACTGAATCAG	CCTAAGTCAGTCACTGAG	CCCAAGTCAGTCACTGTA	0,0
One_RFC2-102	A	G	TCCAGGAGCTGCATTTTGAGTTAAA	AAGGTGGATGACAATGTGTTAGTGT	ATCACGTTGTATTTCTTT	CACGTTGTGTTTCTTT	0,0
One_RFC2-285	A	T	GGATGAGGCTGACAGGTAAGTC	ACAGTCGTTATAGGTACAGGTACACT	CACGACATCTAAGCTGAA	CACGACATCTATGCTGAA	0,0
One_RH2op-395	G	T	GCTGTAGGTCAAACTCGAAGAG	CAGCCTTGTTCAACCCCATTTACTA	TGGGAACATCAITTTTTTAA	TTGGGAACATAAITTTTTTAA	0,0
One_rpo2j-261	G	T	GATTCTGAGATCATACAGTGGATTGGT	GCTTGTCATCTTTCAGCACATACCTA	CACATGTTTTACTCATTTGA	CACATGTTTTACTAATTTGA	0,0
One_sast-211	G	T	TGTACTTAGTCCAATAAGCAATTTCAACAGT	TGGCTAGATTCAATGGTCAACAAA	CATCATTTGCAATTATTG	CATCATTTGAATTATTG	0,0
One_spt30-207	G	T	AGCAITTCAGTTTTGTACATTTACAGTAAAAACA	ACCTACTCGTAATTTTCAGGGCAAAA	AGGGACATCTTACCTCAAAA	AGGGACATCTTACCTCAAAA	0,0
One_srp09-127	T	A	CGGAGCTGGAATGACGACAT	AGGTTGAGCAAAATCCCTCTTTAGAG	CAGCGAAGGATATGCT	CAGCGAAGGTTATGCT	0,0
One_ssrd-135	-	T	TGGAAACTCCTAGTGTACTTCATTCTCA	CGTTCCACGCTCCCTAGAATAGA	CTGCGGCTTTGTCTTG	TGCGGCTTTGTCTTG	0,0
One_STC-410	T	C	CAACACAACATCAACATCATTAAATAAACATTCTG	AACATCCCCGTTTTGACCACCTAT	CCGATGGGTATATTATTATA	CCGATGGGTATATTGTTATA	0,0
One_STR07	G	C	CACACCTGAGGCACAAGCT	GTATGTCTACCAGAGAGGTCAAGGA	ACGCACACTGTCCCT	ACGCACACTCTCCCT	0,0
One_SUMO1-6	C	A	GCACAAGCCAAAAAGTTTTCTCCAT	GGACATAGTTGGAGGCAGACAAAA	CAAGAT[AT]GAAATTGGTTTGC	CAAGATTGAAATTTGTTTGC	0,0
One_sys1-230	T	G	CTACCTGTCTAACAGTGAATGCTAACTT	TGAAACCAATTAAAGCTCTTTGTAGACAA	CAAAGCAAGTGATATAATTAGTG	AAAGCAAGTGATATCTTAGTG	0,0
One_taf12-248	G	T	ACCTTCAATTAGTGGTGGTTACC	ACTAAACGCACAACAGCAAAACG	CCAGACAAAATAAAATTA	CCAGACAAAATAAAATTA	0,0
One_Tf_ex11-750	G	A	AGCAGGTGTAAGCATGTGTACTT	CCTGCTCTGCCTCAACAATGTTAA	CAGGGTCGCTGCAC	CCAGGGTCACTGCAC	0,0
One_Tf_in3-182	A	G	GCCCTTAGCACTTCAGTTGCA	CAGACAGAAACCATTTGATCCGATTC	AACAGAAAGTCTACACTTT	ACAGAAAGTCTGCACTTT	0,0
One_txnlp-401	C	T	GCCAGATCCCTTCAGTTGGA	GGCCATTTCAAAAGGCTGCAT	TGACTGCACTAGTTTAGAC	TGACTGCACTAATTTAGAC	0,0
One_U1003-75	C	T	TCACGAGCCCCAGTCAGA	CGGGTTTCGGTGGTTTAGTATTCTA	AGAGACTACTTCCTTTTTTG	AGAGACTACTTCTTTTTTTG	0,0
One_U1004-183	A	G	GGTGATGACTGCTGTGTTTAATTGC	ACCATCATTAACAGCAATTCTGAGT	AAGTTCCTGTATTTCCT	TCCCTGCATTTCTT	0,0
One_U1009-91	A	G	CTCTGCTTGAAGCTGTTGCTGTT	CGCGTCTGACTCTTCTCT	GTGTTCTGTATGGACCC	TGTTCTGTGTGGACCC	0,0
One_U1010-81	A	G	CAGCCCTCGAGGTAAGT	GTTGAGACAACAAACGTCTACTGT	CACACCAACGTTATGTAGAG	CACCAACGTTGTGTAGAG	0,0
One_U1012-68	C	T	TCTATTACCATACAGGCCAGTACA	CCTTTTGTGTCTTCCAGTCATGTGA	TGACGGGTGTTTCTGTATAA	TGACGGGTGTTCTTGATAA	0,0
One_U1013-108	G	T	TCTGTGCTCTCTCCAGGAT	CGAAACTGAGGAGTGCTCTGA	ACGGAATTCCTGTGTCCT	ACGGAATTCCTTTGTCCT	0,0
One_U1014-74	C	T	TCCCTGCGAGCAACTGTTTT	GGCAGAGACGGCATCCT	TTGACCTGCGCCAGTAT	TTTTGACCTGCACCAGTAT	0,0
One_U1024-197	G	T	CTGAAGTATCTACCGCTCTGT	GGAAACAGATACTCCAGGAGAGATGA	ACCTGACCCAACAAA	ACCTGACACAACAAA	0,1,1
One_U1101	C	A	CTATGACATGTTTTATTTAATAGCCACCAACT	AGTATAGCTAGGGAACCTTTCGATCTT	TGGACGTATGCTATATT	TGGACGTATGTAATATT	0,0
One_U1105	T	A	GCCTTAATAGTGTCTTCTGATCCCTTT	CCCTCTGTTGTCCAGACTCTTAG	CCTGTTTTTTTTAAAGAC	TCCTGTTTTTTTTAAAGAC	0,0
One_U1201-492	A	G	GCTTATGACGGAGAAGAGATGCA	AGGATACTGAAGCCCAGAGACA	AAGACTTCCTCCAGGCTC	ACTTCCCCAGGCTC	0,0
One_U1202-1052	T	C	CGATTTGAGTCTCCAATGGTCTCT	ATTCTATGGTTAAACATCAATTCTATAAAGTCAT	CAAACCTTTTTCATCTACATTTA	ACTTTTTCATCCACATTTA	0,0
One_U1203-175	G	A	CCCGGAGACATACTTGATGCA	GGAGGACCTGCAGGATCAC	CCATAGTTGCTGGGCTT	CTCCATAGTTACTGGGCTT	0,0
One_U1204-53	C	T	GTAACCCCTTCATGTTGGCCATT	CTCCATGTCTGAATGTCCCATCA	ATGCATACACGCTGATGC	ATGCATACACGCTGATGC	0,0
One_U1205-57	A	G	AGTAAATGGTTATTACGTAACGGATAAG	CAGGACAGTCTCCACATTCTAACAGA	AGTTATCATGGTCACTCT	AGTTATCATGGTCTCTCT	0,0,8
One_U1206-108	G	T	CTGAGATGGTGCTTTCTGAGGATA	TGGATGAAAGGGAAATTCTGTCAACA	AACATTGAGCTTCCC	ATAACATTGATCTTCCC	0,0
One_U1207-231	C	T	GGCCAAACTGACAGGGATCTATTAA	GGGTCCAGTCTGTACACCATCTAT	ACATTCTTGGCATTGC	CATTCTTGCATTGC	0,0
One_U1208-67	A	C	ACTTGAATGTCTGTTTCGTAGGTGAT	ACACAGTTGACAGTGGAGCAA	CCCAATGTGATTGTCAC	CCAATGTGCTGTGCAC	0,0
One_U1209-111	C	T	GTCACGTAATCACGAGAAAGATACTAAATGT	TCTGCGTCTCCAGAGAGGTT	CTCACATCGAGATGATC	TCACATCGAAATGATC	0,0
One_U1212-106	A	G	CGTAATGACCTACCACCATATCAGT	TGGCATGACTTTAAACAATCCCCAAAAA	TTTTGACATACAAAAATA	TTTGACATACAGAAAAATA	0,0
One_U1214-107	A	C	CCAAATGTACTCCATGTTGGTTAGC	TGCTGAGTATTAAGCTATATCATTTGAAGTTTT	TAGTGACCTATTAATTTGC	TGACCTATTTCAATTGC	0,0
One_U1215-82	A	C	GTGTGCTGGTTTCGTTGGAGTAG	TCCTCAGAAAGAGGAATACCAAGTTC	AATGAGACAAAGTATTTGGT	AATGAGACAAAGTCTTTGGT	0,1,9
One_U1216-230	A	T	TGGGATCGGACGTCAATAGATTTT	GTAATACAGAGTGAGCGTGATACATTGT	CCTGGCTACTAAGTAAC	CTGGCTACAAAGTAAC	0,0
One_U301-92	T	G	AGCCAGTAGCCGATAATGTTTGTG	CCCCCCCCAAATTTGCTAGCT	CCATGGATTAATAATTT	CCATGGATTAATAATTT	0,0
One_U401-224	C	A	GGGTGGAGACGAACGGATT	GTACGATTTTTTTGTAGCCCCAAGT	CACCTGGAAGGACTGA	ACACCTGGAATGACTGA	0,0
One_U502-167	A	G	GCTTTTGTGCAATAGCTATGTTGCTTA	GCAAAGGTAGGCAGCAGATTG	CTTCTTGATCAATAACG	CTTCTTGATCGATAACG	0,0
One_U503-170	T	G	GATTGAGAAATGCCACGACAAAGAA	GTGATTGGTACATGTCTGTGAGTT	AAGTACTAAAATC[AT]GTTTTACATTG	TACTAAAAATCAGTTGTACATTG	0,0
One_U504-141	C	A	GCTATAGCTCACAGAGGATCCCA	TATTGGCGGGTGAGGGATG	TCAAGGACACAAACAA	TCAAGGACAAAAACAA	0,0

One_UCA-24	C	T	AACTCTGCGTCTGTCTGCTT	TCAGATGGTTCATTATGACAGCAACAGC	CGAACAGGGCTGGATG	CGAACAGGACTGGATG	0,0
One_vamp5-255	C	T	GGTTGACTTTTCTTAACCTTTTAAATCTGTGATATTGT	GCTGAGCTAGTGATGGTACCATTT	TAGGCTCCGTGCTCAGT	TAGGCTCCGTACTCAGT	0,0
One_vatf-214	C	A	TCATTCCTTTGCCTGGAGCATT	GGCATACAGCAAAACAATTCAACCA	TGGTATTACTGTGCATTGAC	ATG[CG]TATTA[AC]TG TTCATTGAC	0,0
One_VIM-569	G	A	TTCTGGGTGGACTCATTGATCAC	ATGCGTTATACCTGTAATCTGCAAGT	AAGTGTTCCATACTCACTATA	AAGTGTTCCATATTCACTATA	0,0
One_ZNF-61	C	A	CCATTCA TGTTCTATT CAGATATATTTTGTGCA	CCTAGCTAGAGCTCAACAATATGCA	CTATGGACATGATCTTT	TTCTATGGACATTATCTTT	0,0
One_Zp3b-49	C	A	TCCTCGTGGTTATAGTTATAAAGATGTCAGT	TTGGCTCTGCACTCGGTTTA	AGGCCCAATCCTT	AGGCCAAATCCTT	0,0

Section 2: Genetic Baseline Expansion

Introduction

Distinct population aggregates of Chinook Salmon (*Oncorhynchus tshawytscha*), steelhead trout (*O. mykiss*), and the species *O. nerka* (Sockeye Salmon and kokanee), have evolved through the cumulative effects of selection and genetic drift (Waples 1991). The homing behavior (philopatry) displayed by Pacific Salmon means that fish typically return to spawn in their natal rearing sites or stream of origin. This distinctive life history attribute can significantly restrict gene flow, shape regional variation, and influence demographics among naturally reproducing populations (Hasler and Scholz 1983; McIssac and Quinn 1988; Quinn *et al.* 1991). Genetic differentiation is most easily resolved among populations that are geographically distant, where degree of gene flow is generally correlated with relative migration distances and adjacency in stream networks. However, local adaptations and the distribution of suitable spawning habitat within stream networks may influence finer (regional) scale genetic structure among watersheds in close proximity (Beacham *et al.* 2006; Matala *et al.* 2012). The natural phenomenon of immigration or straying (a homing miscue) buffers the loss of genetic diversity in salmon populations (Milner and Bailey 1989), but the rate of straying exhibited by wild fish is generally low (Quinn 1993; Heard *et al.* 1995) and genetic structure between populations may persist despite moderate gene flow from straying (e.g., Neville *et al.* 2007). Some evidence indicates that hatchery-origin fish exhibit a higher rate of straying which may be affected by changes in fish passage protocols, transport through the hydro system, artificial rearing practices, or inadequate acclimation (imprinting to natal waters by juvenile salmon). An elevated rate of immigration between populations may erode local adaptations, and lead to changes in spatial and temporal variability within and/or among populations (Hess and Matala 2013; Hess *et al.* 2016a; Matala *et al.* 2017).

In the Columbia River Basin, Chinook Salmon have been studied extensively (e.g., Waples *et al.* 2004; Beacham *et al.* 2006; Narum *et al.* 2008b; Matala *et al.* 2011; Hecht *et al.* 2015), as have steelhead trout (Winans *et al.* 2004; Currens *et al.* 2009; Blankenship *et al.* 2011; Narum *et al.* 2011; Matala *et al.* 2016). The scope of Sockeye Salmon and Coho Salmon genetic monitoring has been comparatively limited but has received greater attention in recent years (Gustafson *et al.* 1997; Kozfkay *et al.* 2008; Iwamoto *et al.* 2012; Galbreath *et al.* 2014). Continued monitoring and evaluation of the genetic structure among salmon populations in the Columbia River Basin has guided managers in establishing and maintaining primary conservation units to protect fisheries resources. The delineation of such conservation units, including distinct population segment (DPS), evolutionarily significant unit (ESU), major population group (MPG), and viable salmonid population (VSP) is guided by a core set of criteria, including population ecology and viability, ancestry and descent, reproductive isolation, and genetic structure and local adaptation (Fraser and Bernatchez 2001; Fraser *et al.* 2011). Although an understanding of adaptive variation is critical to proper salmon management, the majority of genetic information available to managers is based on neutral genetic variation. Landscape genetics is an approach aimed at describing population differentiation relative to features in an organism's environment (Segelbacher 2010; Latch *et al.* 2011; Sepulveda-villet & Stepien 2012; Matala *et al.* 2014). Landscape genetics explores population differentiation relative to features in the environment such as migratory barriers (e.g., dams), or heterogeneous habitats such as variation in local climates or temperatures (Dionne *et al.* 2008; Narum *et al.*

2008a; Micheletti et al. 2017). Although local adaptation may be inferred from landscape genetics (Olsen et al. 2010; Blankenship et al. 2011), inferences based primarily on neutral genetic differentiation risk incorrectly identifying the underlying processes affecting population distinctions (Funk et al. 2012; Landguth & Balkenhol 2012). Techniques such as outlier detection methods, and genome wide association studies (GWAS) based on DNA sequence variation provide evidence of non-neutral population structure or adaptive variation (markers associated with run timing; Hess et al. 2016b). Such applications in genetic monitoring allow a more resolved understanding of genetic differentiation beyond what can be concluded from neutral loci alone (Narum et al. 2010b; Matala et al. 2011; Ackerman et al. 2012, Bourret et al. 2013). Putative non-neutral population differentiation can then be interpreted in the context of contemporary risks and vulnerabilities (e.g., climate change) for salmonid populations in the Columbia River Basin, revealing highly correlative relationships between genetic variation and the physical environment (see Limborg et al 2011). This additional information may ultimately influence conservation criteria for delineating populations across diverse landscapes.

Project objectives, time line and harvest management questions:

Objective two of project #2008-907-00 (Genetic Assessment of Columbia River Stocks) describes efforts to evaluate genetic diversity among populations that will inform managers in the areas of harvest monitoring, and conservation monitoring. Our approach involves the collection, analysis, interpretation and distribution of genotypic data. These data are being compiled as species-specific reference baselines for characterizing Chinook Salmon, steelhead trout, and *O. nerka* population structure specific to the Columbia River Basin. Baselines were initially created from genotypes at single nucleotide polymorphism (SNP) loci, which are highly prolific in the genome and provide substantial coverage for linkage analyses (Moen et al. 2008). SNPs are amenable to superior high throughput capabilities and are relatively easily amplified and scored compared to other types of genetic markers, even with poor quality tissue (DNA) sources (Campbell and Narum 2008). Because SNPs are commonly found within or adjacent to coding and regulatory regions of a genome, corresponding allelic diversity and allele frequency variation are likely to be informative for understanding non-neutral influences (i.e. selection and local adaptation) on observed population structure. Large numbers of highly informative SNP loci have been discovered through our ongoing efforts using a next generation sequencing technology known as restriction-site associated DNA (RAD) sequencing (Miller et al. 2007; Baird et al. 2008; Hecht et al. 2013). Our two primary objectives for utilizing SNP baselines to monitor salmon species in the Columbia River are 1) genetic stock identification (GSI) of natural-origin stocks, and 2) parentage based tagging (PBT), a large-scale, non-lethal tagging technology for monitoring and evaluating hatchery stocks. The collaborative, inter-agency application of GSI continues to provide invaluable monitoring capabilities to understand relative stock proportions in sport, commercial and tribal harvests, as well as monitoring of stock specific run-timing at Bonneville Dam, Lower Granite Dam and other fish weirs in the basin. Moreover, GSI is being used concordantly with PBT to monitor trends in hatchery production, harvest of hatchery fish, and population attributes of specific hatcheries (e.g., stray rates, survival/mortality, migratory behavior, hatchery/wild interactions). Additionally, our genetic baselines are being used to characterize populations in archival studies, to inform efforts to reintroduce fish into extirpated regions within historic ranges, and in domestication studies. Objectives will be ongoing and our most recent results will be reported each year. As new genetic techniques are developed they will be applied to our objectives and data will be routinely uploaded to the

FishGen.net database (<http://www.fishgen.net/home.aspx>) as a repository for data sharing and collaboration.

Methods

Baseline sampling and protocols:

Our previously established baselines, comprised of putatively neutral SNPs (e.g. 180 loci for *O. mykiss*), have been well characterized and have been used extensively for genetic stock identification (GSI) as described in Hess et al. (2015) and Hasselman et al. (2016). Our most recent efforts focus on expanding genetic characterizations throughout the basin that will provide information about adaptive potentials and natural selective forces contributing to stock structure. Next generation sequencing technologies in genotyping (RAD) continue to be employed by the CRITFC genetics lab in order to expand SNP panels for Chinook Salmon, Coho Salmon, Sockeye Salmon, steelhead trout and Pacific lamprey (Hess et al. 2016c). Methods for genotyping by sequencing (GT-seq) are described in Campbell et al. (2015), and for RAD sequencing (Sbf1 restriction enzyme protocol) see Monitoring Methods #4144, owner: Nathan Campbell. SNP discovery using the RAD sequencing technique is described in Chapter-1 of this report. Detailed laboratory methods are available in Hess et al. (2012) and in Monitoring Methods: <https://www.monitoringmethods.org/Protocol/Details/230> (ID#230; owner Matthew Campbell). The program STACKS was used to identify and quality-filter SNPs from raw Illumina sequence data (Catchen et al. 2011). High-quality SNPs were then used to assess population structure based on principal component analyses (PCA) and pairwise genetic distance displayed in neighbor joining (NJ) trees using the ‘adeigenet’ package in R (Jombart & Ahmed 2011) or in GenAlEx v6.5 (Peakall and Smouse 2006).

Expansion and status of reference baselines for GSI:

Our three primary goals for expanding, maintaining, and evaluating each species-specific baseline are threefold. First, we used genetic stock identification (GSI) analyses for monitoring of fishery returns through the migratory corridor of the Columbia River, including harvest GSI in the lower Columbia River, and fish passage GSI at Bonneville and Lower Granite dams (see sections 3 & 4 of this report). Secondly, PBT broodstock sampling and genotyping of Columbia River Basin hatcheries has been updated to include return year 2016 for continued evaluation of hatchery stock composition in various fisheries and to monitor hatchery impacts on wild populations. The sequence/genotypic data are also being applied in various analyses to evaluate selection, including investigations of landscape genetics, and adaptive differentiation among populations. The GT-seq primer pools developed from RAD-seq data are currently being used for all high throughput genotyping projects, including 5 target species: steelhead trout – 268 SNP loci plus sex determination marker, Chinook Salmon -298 SNP loci plus sex determination marker, Coho Salmon - 257 SNP loci, Sockeye Salmon - 363 SNP loci, and Pacific Lamprey - 308 SNP loci (Hasselman et al. 2016). Currently we do not utilize reference baselines for genetic stock assessment of either Coho Salmon or Pacific Lamprey, and in 2017 there were no updates to the reference populations in our baseline for Chinook Salmon based on the expanded SNP panels. The most up to date GSI marker panel used for stock assessment of Chinook Salmon is described in Hess et al. (2016c). For *O. nerka*, we completed efforts to expand our current SNP panel from 92 SNPs to 363 SNPs, (Hasselman et al. 2016), which was used to update the reference baseline for *O. nerka*. Updates to the reference baseline based on the expanded SNP marker panel for steelhead trout is described in the three sections that follow.

Oncorhynchus mykiss GT-seq SNP panel expansion

The ability to genetically assign individual fish to geographic region-of-origin facilitates better management of distinct groups or populations. In past years, our marker panels (180 SNPs) for *O. mykiss* have been employed to successfully assigned individual steelhead to 13 distinct reporting groups in the Columbia River Basin: Quinault, Lower Columbia, Skamania, Willamette, Big White Salmon, Klickitat, Yakima, Upper Columbia, South Fork Clearwater, Upper Clearwater, Middle Fork Salmon, South Fork Salmon, Upper Salmon (Figure 1). However, the 14th reporting group, termed Mid Columbia-Grande Ronde-Imnaha-Lower Snake-Lower Clearwater-Lower Salmon (MGILCS), is less distinct because it spans multiple sub-basins (Hess et al. 2016). Thus, any genetic assignments to MGILCS are less informative than other reporting groups since individuals may have originated from one of many far-off or non-adjacent tributaries within MGILCS. Efforts to expand the reference baseline for *O. mykiss* in 2017 were focused on discovery of additional genetic markers that would provide better resolution of the populations within MGILCS, allowing separation of smaller reporting groups. This was achieved by employing a Restriction site-associated DNA (RAD) method (Micheletti *et al.* 2017). Populations representing 21 localities within the MGILCS reporting group were scrutinized as groups based on adjacency in the stream network and greatest genetic similarity (*i.e.*, those with high F_{ST} and greatest allele frequency differences) using 24,526 single nucleotide polymorphisms (Appendix 1; Micheletti *et al.* 2017). To select the most informative SNPs, a principle component analysis (PCA) was iterated in the R package *adeigenet* (Jombart 2008) with different combinations of candidate SNPs from the 24,526 RAD markers, until sufficient (*i.e.* greatest) differentiation was observed between spatially distinct tributaries within MGILCS

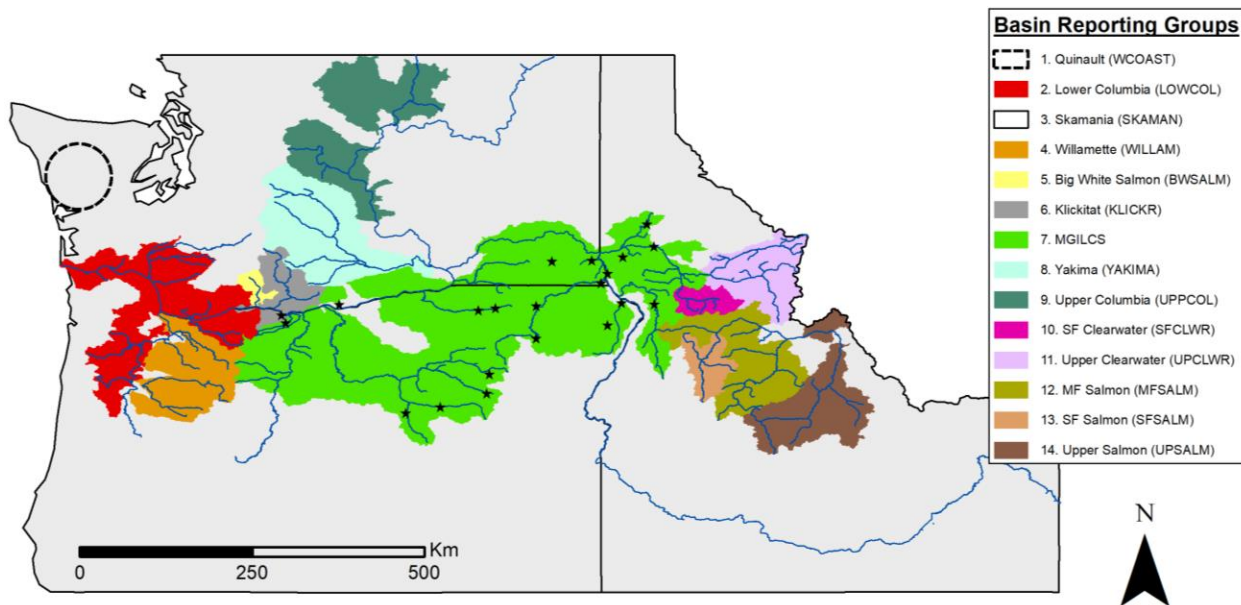


Figure 1: Reporting groups that were used in the previous GT-seq panel. 21 populations within MGILCS from the Micheletti *et al.* 2017 RAD study are represented by stars.

(Figure 2). Each combination of SNPs was double cross-validated (75% training, 25% test) to avoid grading bias (Kearns and Ron 1999). With the goal of reducing the number of informative markers below 100, an initial subset of 200 RAD markers that differentiated the 21 localities were selected based on the top average squared loading scores for the first two principle component axes. Next, pair-wise F_{ST} was performed between localities using LOSITAN (Antao *et al.* 2008) and the top 200 RAD markers were further subset to 100 RAD markers which had the highest average pair-wise F_{ST} . After PCA iterations, a group of 63 SNPs were shown to produce high-resolution differentiation between 7 reporting groups within the larger MGILCS reporting group (Figure 3).

To ensure that the newly developed 63 markers could successfully identify the reporting group of origin, genotypes were simulated for 56 localities in the Columbia River Basin, representing 14 reporting groups (Micheletti *et al.* 2017), among which the MGILCS reporting group was broken up into 7 smaller reporting groups. We used the R package *GPoppin* (Appendix 1; Micheletti *in prep*) to simulate genotypes for each locality in a process that adjusts for Hardy-Weinberg and linkage disequilibrium. For each locality, the simulated genotypes from allele frequency distributions of 100 individuals were generated using the 180 SNP markers from

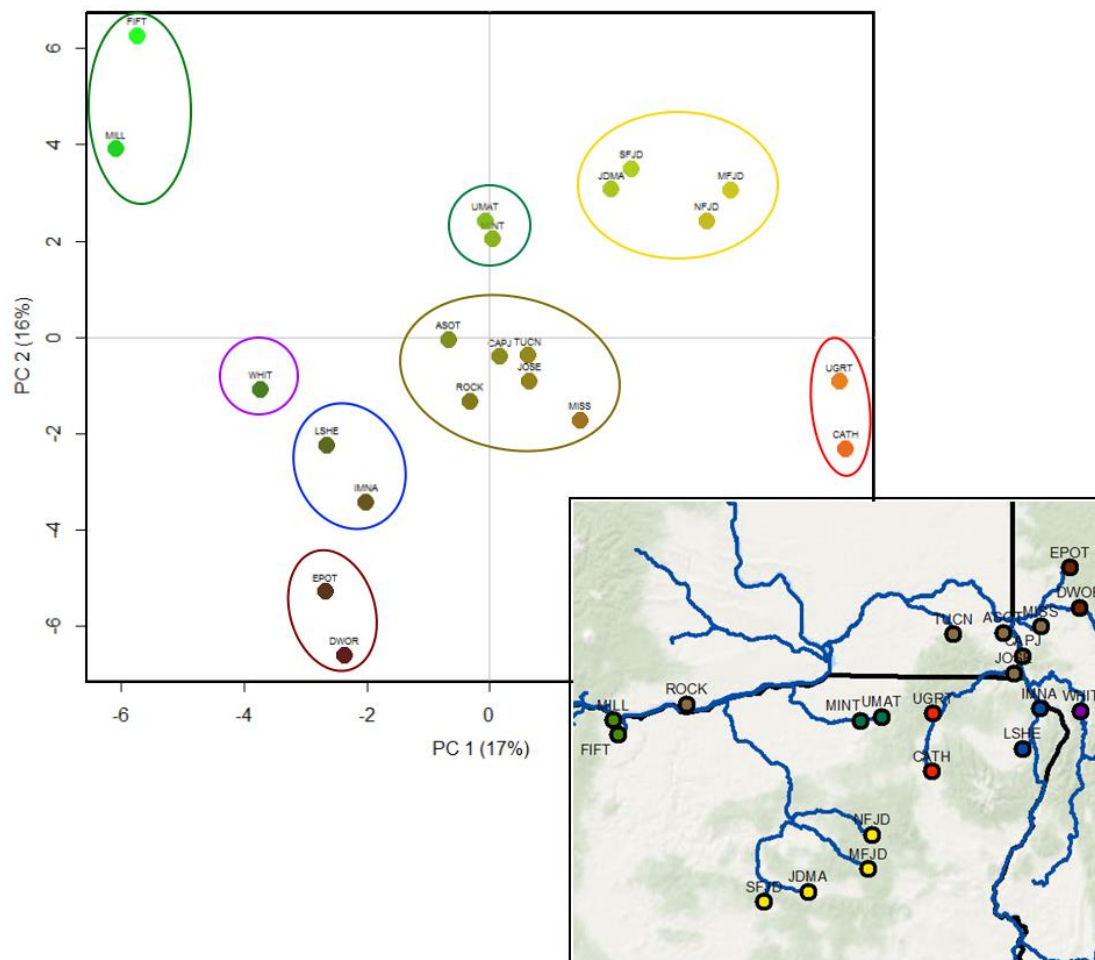


Figure 2: Principle component analysis (PCA) illustrating differentiation between distance-isolated tributaries using allele frequencies of 63 SNP markers from 21 MGILCS populations. (Micheletti *et al.* 2017).

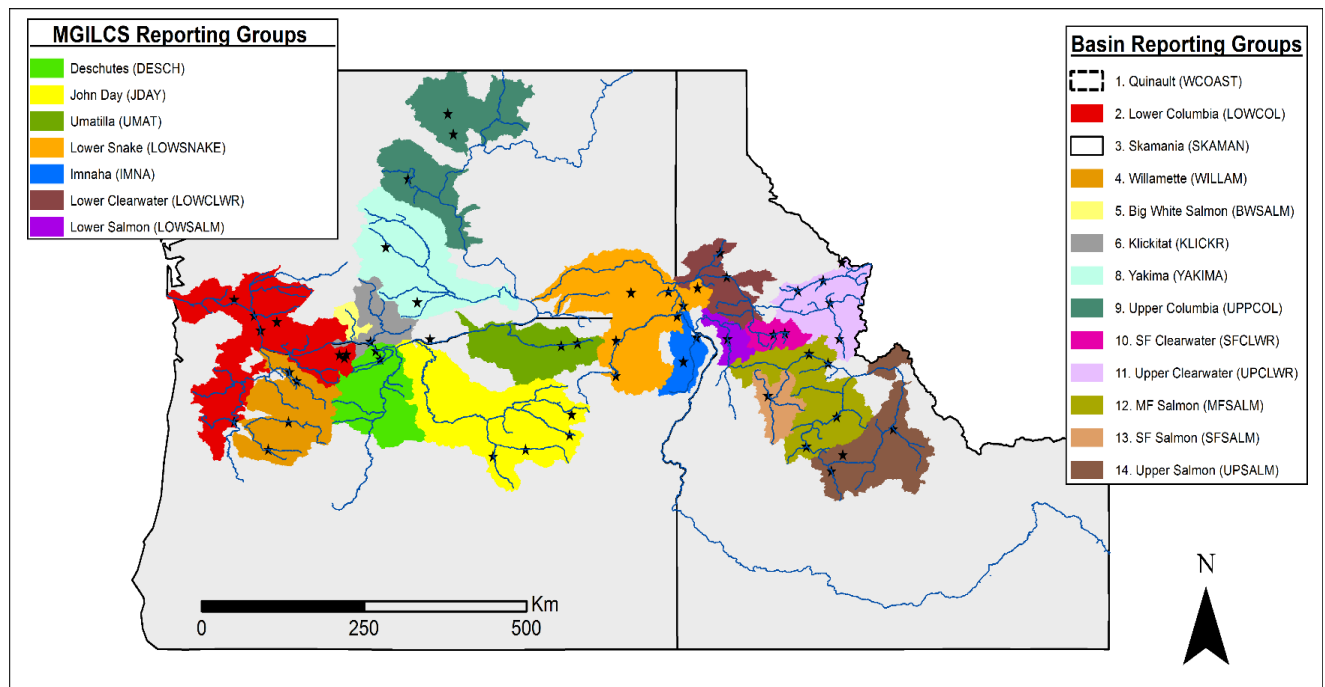


Figure 3: Expanded reporting groups after incorporating the new 63 genetic markers. All populations from the RAD study are displayed as stars.

our previous GT-seq panel (without the new 63 SNPs) as described in Hasselman *et al.* (2016), and in an updated GT-seq panel that included the new 63 SNPs in addition to the existing marker panel. Using *GENECLASS2* (Piry *et al.* 2004), simulated individuals were then assigned to the most likely locality-of-origin in a reference baseline of genotypes from individuals of known origin in each of the 56 localities.

Marker expansion for O. mykiss based on environmental associations

Populations of *O. mykiss* from across the Columbia River Basin experience regionally and/or locally variable environmental (e.g., habitat) conditions, such as air temperature, water temperature, precipitation, stream flow, elevation, and migration distance (Micheletti *et al.* 2017). Variation in environmental conditions can be strongly correlated with specific alleles at specific loci, where certain alleles favor survival under certain habitat conditions. To understand the influence of environment on genetic variation among *O. mykiss* populations, we identified SNPs associated with a variety of environmental factors as described in Micheletti *et al.* (2017). Use of such loci for characterizing populations that experience variable habitat conditions may help predict the optimal conditions for particular groups of fish and will inform management decisions when environmental conditions are expected to change (e.g., climate change). We used gene-environment association (GEA) tests to identify markers displaying significant association between allele frequency (among 24,526 RAD markers) and one or more of 21 uncorrelated environmental variables that could potentially influence adaptation (Micheletti *et al.* 2017). Measured environmental variables span three broad categories: temperature-related, precipitation-related, and topographically-related. These include the full suite of climate variables from the Bioclim database ('bio1' – 'bio19'; Hijmans *et al.* 2005), land cover related variables from the National Land Cover Database (Homer *et al.* 2015), stream temperatures from the NorWest database (Isaak *et al.* 2017), elevation from the national elevation dataset (USGS),

and wind velocity and solar radiation from the Vaisala weather database (Vaisala, Vantaa, Finland). Additional variables were calculated directly in ArcGIS 10.3.1 from a digital elevation map, such as slope, roughness, and heat load index. Finally, stream network variables from the National Hydrography Dataset were extracted, such as migration distance to the sea, stream order, and number of dams (USGS).

We used a suite of GEAs tests that incorporate different statistical methods to correct for population structure (i.e. false associations), including Bayenv2 (Günther and Coop 2013), latent factor mixed models (LFMM; Frichot *et al.* 2013), and autoLM (Micheletti *et al.* 2017). Bayenv2 uses a covariance matrix to estimate the neutral population structure (Günther & Coop 2013), where Bayes factors are calculated for each locus to compare models in which an environmental variable has a linear effect on allele frequency to a model in which allele frequency is explained by the covariance matrix alone. LFMM uses a Bayesian mixed-model that employs a principal component to determine background level of population structure. Environmental variables are used as fixed effects and latent factors as random effects to determine locus-environment associations (Frichot *et al.* 2013). Finally, AutoLM uses a linear mixed-effects model implemented by ‘lme4’ R package (Bates *et al.* 2014) to find significant correlations between allele frequencies and environmental measurements while simultaneously using spatial autocorrelation as a covariate in the model. Using recommended thresholds for significance, only those markers with at least two significant GEA results among the three test methods were considered to be associated with an environmental variable. In addition, significant markers were “blasted” (ncbi.org) to determine the location of SNPs markers within or near particular genes.

Run-timing marker expansion

The run timing of *O. mykiss* as they return to freshwater to spawn is a distinct trait involving maturation. Winter-run fish, referred to as ocean maturing, often cannot be easily or reliably differentiated from summer-run fish (stream maturing) based on physical appearance alone. This is largely due to the protracted return time of summer-run fish, which may reside in the freshwater environment for up to year as they reach reproductive maturity (e.g., gonadal development). Two SNP markers in proximity to the gene *GREB1L* that are associated with maturation or run-timing, were incorporated into our GT-seq panel for *O. mykiss*, and have been informative for differentiating steelhead run-types in the Klickitat River (Hess *et al.* 2016b). Despite the value of these markers, which display large allele frequency differences between summer-run and winter-run fish, we were prompted to continued efforts to discover additional run-timing markers with the intent of improving confidence in assignment tests to determine run-timing. Additional run-timing markers were identified using pooled sequencing (Pool-seq) of individuals sampled from the Klickitat and Kalama rivers, where “known” winter-run or summer-run origin of fish was based on documented spawn time. The DNA from 50 individuals with the same run-type and from the same locality was mixed and sequenced as a group. DNA was extracted using a Chelex extraction method (Sweet *et al.* 1996). The contribution of each individual within a pool was normalized with a Tecan M200 (Tecan, Männedorf, Switzerland). Specifically, DNA concentrations had to be within two standard-deviations of the mean. Sequencing followed a NEBNext enzymatic fragmentation protocol as follows. Pooled samples were fragmented using NEBNext dsDNA fragmentase. Pools were then cleaned using a Qiagen MinElute, NEBNext end prep was used to repair the ends of fragmented sequences, and NEBNext adaptors for Illumina were ligated to repaired sequences. Size selection was performed for an average ligated sequence of 500 bp along with PCR amplification, and PCR product

cleaning using AMPure XP beads. Prior to sequencing, the quality of each reaction was confirmed using quantitative PCR.

Because individuals are mixed using the Pool-seq method, it is not possible to identify individual genotypes. However, population allele frequencies across a high proportion of the organism's genome leads to a high likelihood of finding SNPs of interest associated with specific phenotypes (Schlötterer *et al.* 2014). Four Pool-seq libraries (Kalama-winter, Kalama-summer, Klickitat-winter, Klickitat-summer) were aligned to the rainbow trout genome and processed using the *PoolParty* pipeline (Micheletti and Narum *submitted*). This pipeline performs quality filtering of data and identifies regions of the genome that are associated with particular phenotypes. A sliding-window F_{ST} analysis, pair-wise F_{ST} analysis, and a Cochran–Mantel–Haenszel (CMH) test for consistent allele frequencies were used to determine if *GREB1L* was the gene of major effect, and if markers in proximity to the gene are informative for differentiating summer and winter run fish (Kofler *et al.* 2011).

Reference baseline update for Sockeye Salmon

Quality filtering of RAD sequencing data for SNP discovery in *O. nerka* resulted in >6400 SNPs that were selected for further screening to evaluate maximum informativeness. Laboratory methods are identical to those for other species previously described in this report. After the quality filtering phase of the analysis primers were designed for 382 informative SNPs (see section 1 this report) that exhibit a strong ability to differentiate major population groups of *O. nerka* within the basin (Hasselman *et al.* 2016). Of these, 364 SNPs provided sufficient data for evaluating the reference baseline for *O. nerka*. The current baseline is consistent with the previous baseline for GSI that included a 93 SNP panel, except for the addition of a population sample from Dworshak Reservoir in the Clearwater Subbasin of the Snake River (latitude 46.5160, Longitude -116.2954; Table 1).

Statistical Analysis:

We evaluated the distinctiveness of each population in the reference baseline using assignment tests. Individual assignment likelihood scores (LS) were generated using GENECLASS2 (Piry *et al.* 2004), implementing the Bayesian method of Rannala and Mountain (1997) in a leave-one-out jackknife procedure. The assigned origin of each individual was the population with highest corresponding GSI likelihood score from among the top five ranked scores. The rate of self-assignment was defined as the proportion of fish of known origin that genetically assigned to that specific population. The program ARLEQUIN version 3.5 (Excoffier *et al.* 2010) was used to calculate pairwise population F_{ST} to evaluate among group variation. A pairwise matrix of Nei's standard genetic distance (Nei 1972) and an un-rooted neighbor-joining (NJ) tree were generated using PHYLIP version 3.68 (Felsenstein 2008). Pairwise genetic distances generated in GenAlEx version 6.5 were used to display population clustering patterns in a principal coordinate analysis (PCoA) plot.

Table 1: The reference baseline for genetic stock assessment of *O. nerka* in the Columbia River Basin. The provided information includes a population ID#; (*) are kokanee hatcheries, anadromous sockeye (S) or resident kokanee (K) life history (? – unverified), sample size, the number and proportion of individuals in each population that self-assigned to known origin along with a corresponding likelihood score (LS), and the next most likely population of origin for mis-assignments (ID and %n).

ID	origin	region	Subbasin/river	life history	n	self-assignment			next likely	
						#	%	LS	ID	%n
1	Osoyoos Lake	Col.	Okanogan	S	185	185	1.00	100.0	---	---
2	Lake Wenatchee	Col.	Wenatchee	S	185	185	1.00	100.0	---	---
3	Redfish Lake	Snake	Sawtooth	S	81	81	1.00	100.0	---	---
4	Lake Billy Chinook	Col.	Deschutes	?	49	46	0.94	99.4	8	0.06
5	*Meadow Cr.	Col.	B.C.	K	49	18	0.37	82.2	14	0.57
6	Odell Lake	Col.	Deschutes	K	40	40	1.00	99.9	---	---
7	Suttle Lake	Col.	Deschutes	K	45	36	0.80	98.3	8	0.13
8	*Wizard Falls	Col.	Deschutes	K	50	45	0.90	99.1	7	0.08
9	Cougar Cr.	Col.	Lewis	K	29	18	0.62	95.5	5, 14	0.17
10	*Speelyai	Col.	Lewis	K	50	48	0.96	98.8	6, 9	0.02
11	*Lake Whatcom	Col.	Puget Sound	K	49	49	1.00	100.0	---	---
12	Gold Cr.	Col.	Yakima	K	43	43	1.00	99.2	---	---
13	N. F. Tieton R.	Col.	Yakima	K	34	33	0.97	98.9	7	0.03
14	Dworshak Reservoir	Snake	Clearwater	K	112	88	0.79	89.6	5	0.20
15	Wallowa Lake	Snake	Grand Ronde	K	22	20	0.91	98.5	6, 16	0.05
16	Wallowa R.	Snake	Grand Ronde	K	40	38	0.95	99.2	4, 9	0.03
17	Alturas Lake	Snake	Sawtooth	K	40	40	1.00	99.2	---	---
18	Fishhook Cr.	Snake	Sawtooth	K	76	76	1.00	99.7	---	---
19	Stanley Lake	Snake	Sawtooth	K	22	22	1.00	100.0	---	---
20	Warm Lake	Snake	S. F. Salmon	K	38	38	1.00	100.0	---	---

Parentage Based Tagging (PBT) update

PBT began with Chinook Salmon and steelhead hatchery stocks in the Snake River basin of Idaho (2008-present; Steele et al., 2013; Steele et al., 2015). However, we have expanded PBT coverage (Figures 4 & 5, Appendices 2 & 3) to include Chinook Salmon, steelhead, and Coho Salmon broodstocks in all hatcheries above Bonneville Dam using expanded SNP panels of 299 loci for Chinook Salmon, 379 loci for steelhead trout and 257 loci for Coho Salmon. Each year the expansion effort is integrated with existing Snake River PBT baselines as data comes available (Appendices 4).

Adopting PBT to the broader Col. River basin facilitates our ability to genetically track millions of salmonids and provide opportunities to address a variety of parentage-based research and management questions, including stock contributions to fisheries (Byrne et al., 2015), estimates of stock-specific abundance and run-timing at dams (Hess et al., 2016c; Vu et al. 2015), and use of thermal refugia during migration (Hess et al., 2016a).

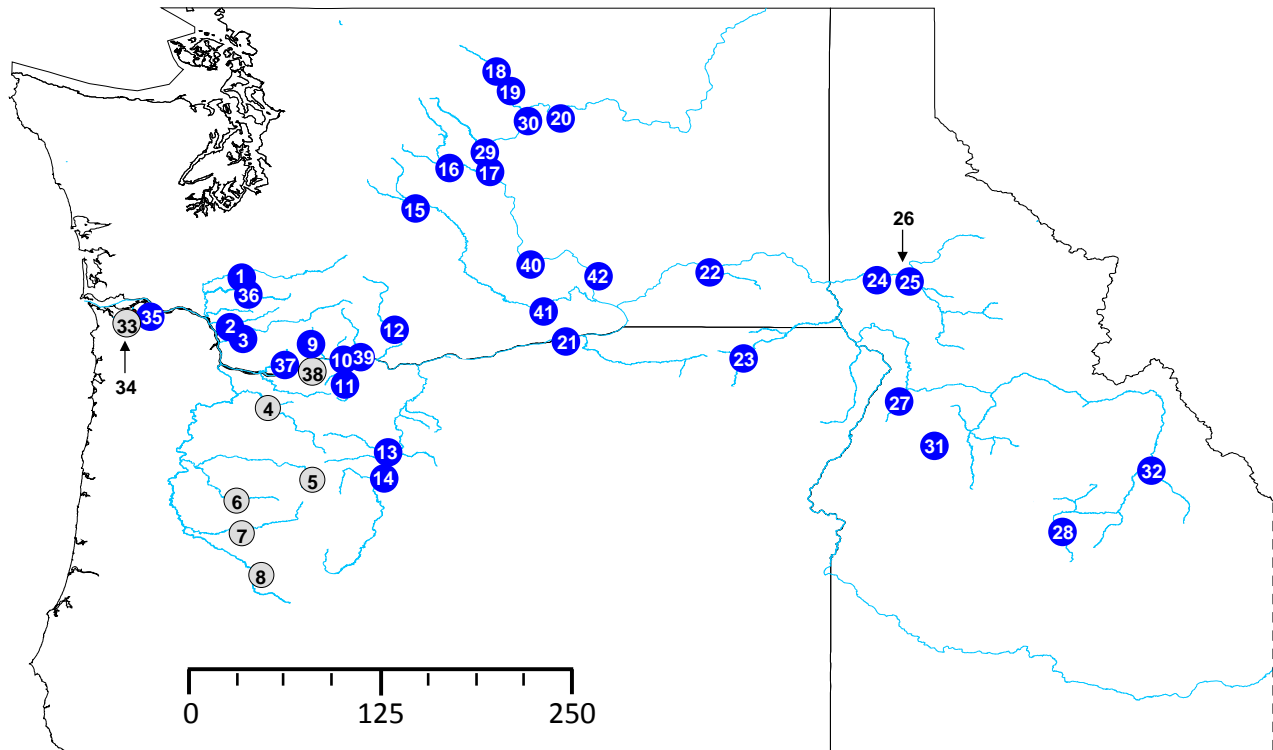


Figure 4: Spring Chinook Salmon and summer/fall Chinook Salmon PBT hatcheries. Numbers correspond to map ID and hatchery descriptions (Appendix 2). Gray circles indicate that a hatchery is not part of the PBT baseline and participation is pending.

The PBT tagging technology has been implemented through annual hatchery broodstock sampling to create a temporally structured parental genotype baseline. As adult fish return to participating hatcheries in the Col. River basin, broodstock are sampled by collection of fin tissue during hatchery spawning. Required data for PBT sampling includes a hatchery record of phenotypic sex and spawn date. Additional and optional information was collected at some hatcheries when resources allowed, including fork length, and mated cross records of male and female broodstock individuals. The PBT baseline was expanded to include spawn years 2012 to 2016, including $n=28,824$ spring Chinook Salmon, $n=81,640$ summer/fall Chinook Salmon, $n=6,938$ steelhead trout, and $n=7,0799$ Coho Salmon. DNA was extracted using modified Chelex extractions and Qiagen DNeasy 96 kits. Extracted genomic DNA was genotyped at 299 SNP loci for Chinook Salmon, 379 SNP loci for steelhead trout, and 257 SNP loci for Coho Salmon using a GTseq protocol: (<https://www.monitoringresources.org/Document/Method/Details/5446>). Hatchery offspring that are subsequently sampled either as juveniles or adults (e.g., in a fishery) are then PBT assigned back to spawned parents which provides the individual age and specific hatchery of origin for each offspring.

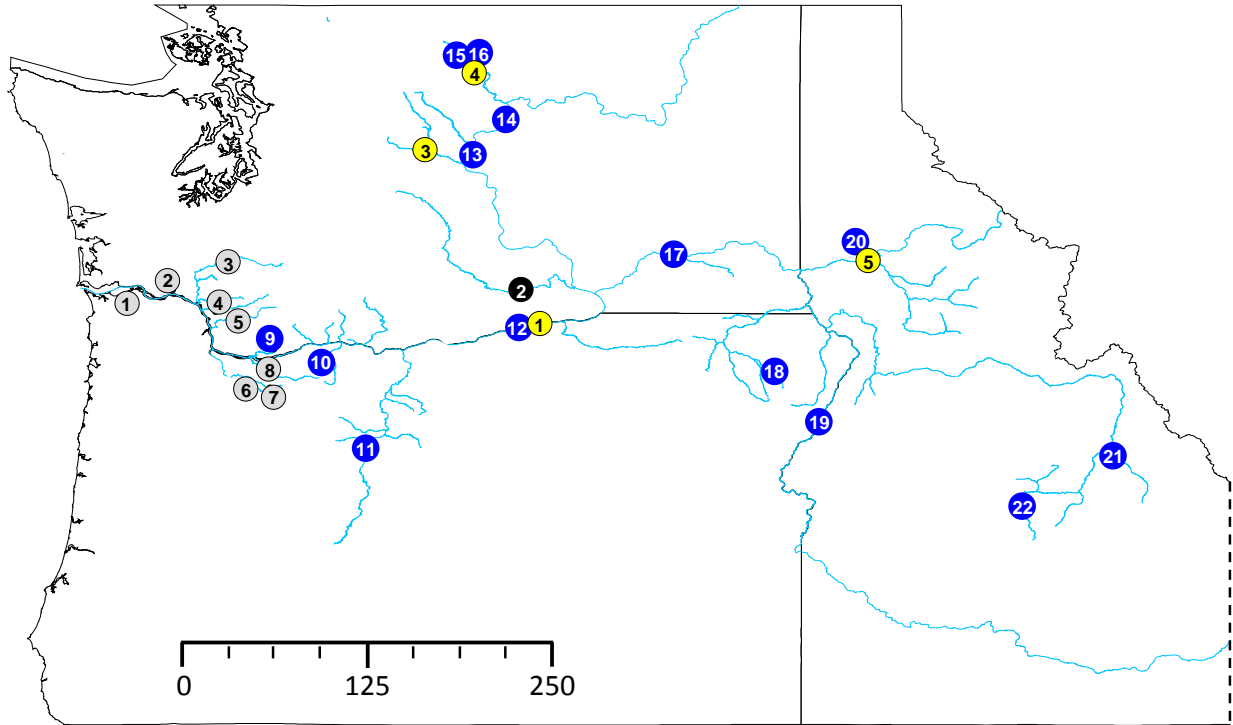


Figure 5: Steelhead and Coho Salmon PBT hatcheries. Numbers correspond to map ID and hatchery descriptions (Appendix 3). Gray circles indicate that a hatchery is not part of the PBT baseline and participation is pending. Blue circles represent steelhead hatcheries currently in the PBT baseline, while gray circles are hatcheries pending participation. Yellow circles represent Coho Salmon hatcheries where tissues have been collected for the PBT baseline, while black circles are pending.

Results

O. mykiss baseline: reporting group and marker expansion

The top 63 RAD markers initially differentiated 8 spatial clusters based on allele frequencies within the MGILCS reporting group: Deschutes, John Day, Umatilla, Lower Snake, Imnaha, Lower Clearwater, Lower Salmon, and Grande Ronde (Figure 2). However, genetic assignments using the new marker panel demonstrated ambiguity between the Upper Grande and Lower Snake regions and thus these regions were combined into a single reporting group called Lower Snake. Using simulated genotypes, the accuracy of assigning individual fish to known origin (i.e. self-assignment) was 91.9% for the existing steelhead SNP panel (Figure 6), and 98.2% after incorporating 63 new RAD markers (Table 2). The substantial increase in assignment accuracy confirmed a high probability of correctly assigning fish that originate from MGILCS to 7 smaller reporting groups within MGILCS (Figure 7).

Table 2: List of 63 genetic marker names added to the *O. mykiss* GT-seq panel that effectively break the MGILCS reporting group into smaller reporting groups.

Omy_RAD2674-44	Omy_RAD1186-59	Omy_RAD24287-74	Omy_RAD43694-41
Omy_RAD12871-35	Omy_RAD1751-18	Omy_RAD24343-29	Omy_RAD46672-27
Omy_RAD12925-52	Omy_RAD2567-8	Omy_RAD25042-68	Omy_RAD49111-35
Omy_RAD14666-65	Omy_RAD2976-26	Omy_RAD25266-23	Omy_RAD49827-67
Omy_RAD14881-63	Omy_RAD3209-10	Omy_RAD25907-57	Omy_RAD50632-21
Omy_RAD16027-52	Omy_RAD7016-31	Omy_RAD28236-38	Omy_RAD57916-29
Omy_RAD30503-56	Omy_RAD9408-71	Omy_RAD29352-6	Omy_RAD59950-44
Omy_RAD34844-68	Omy_RAD12439-64	Omy_RAD30243-74	Omy_RAD65959-69
Omy_RAD39254-12	Omy_RAD13073-16	Omy_RAD32139-58	Omy_RAD73963-73
Omy_RAD42888-53	Omy_RAD14269-30	Omy_RAD33122-47	Omy_RAD76060-20
Omy_RAD63808-66	Omy_RAD14541-72	Omy_RAD35149-9	Omy_RAD86706-72
Omy_RAD62138-53	Omy_RAD15709-53	Omy_RAD37816-68	Omy_RAD93580-37
Omy_RAD95256-58	Omy_RAD17849-16	Omy_RAD38406-19	Omy_RAD70874-8
Omy_RAD366-7	Omy_RAD19340-24	Omy_RAD40641-58	Omy_RAD81818-40
Omy_RAD619-59	Omy_RAD20917-11	Omy_RAD41594-34	Omy_RAD92398-62
Omy_RAD739-59	Omy_RAD23354-66	Omy_RAD43573-37	

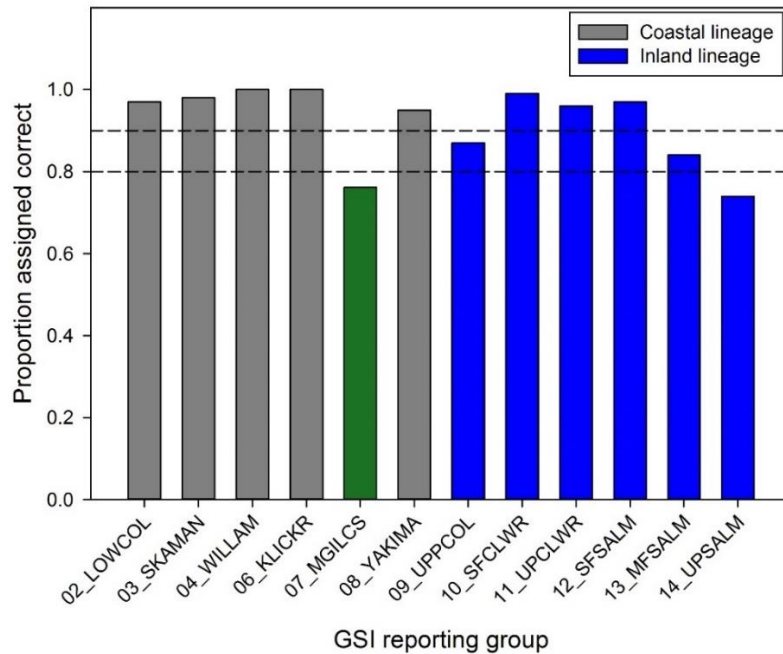


Figure 6: Reporting group assignment success of individuals simulated based on allele frequency distributions with previous 180 GT-seq panel. Assignments to the overall MGILCS reporting group fall below the 90% correct assignment threshold (green).

In tests of environmental association, 8691 RAD markers were determined to be significant on the basis of one or more previously defined GEA tests using recommended thresholds. However, only 63 markers showed significant association based on two or more GEA

tests results that met score thresholds (Appendix 5; p -value < 0.01). These 63 markers were associated with 12 unique variables including water temperature, air temperature, slope, head load index, stream order, canopy cover, elevation, number of dams, migration distance to ocean, precipitation, geological breaks, and isothermality (Appendix 5). Many of these SNPs illustrated near-perfect linear relationships with environmental variables (Figure 8).

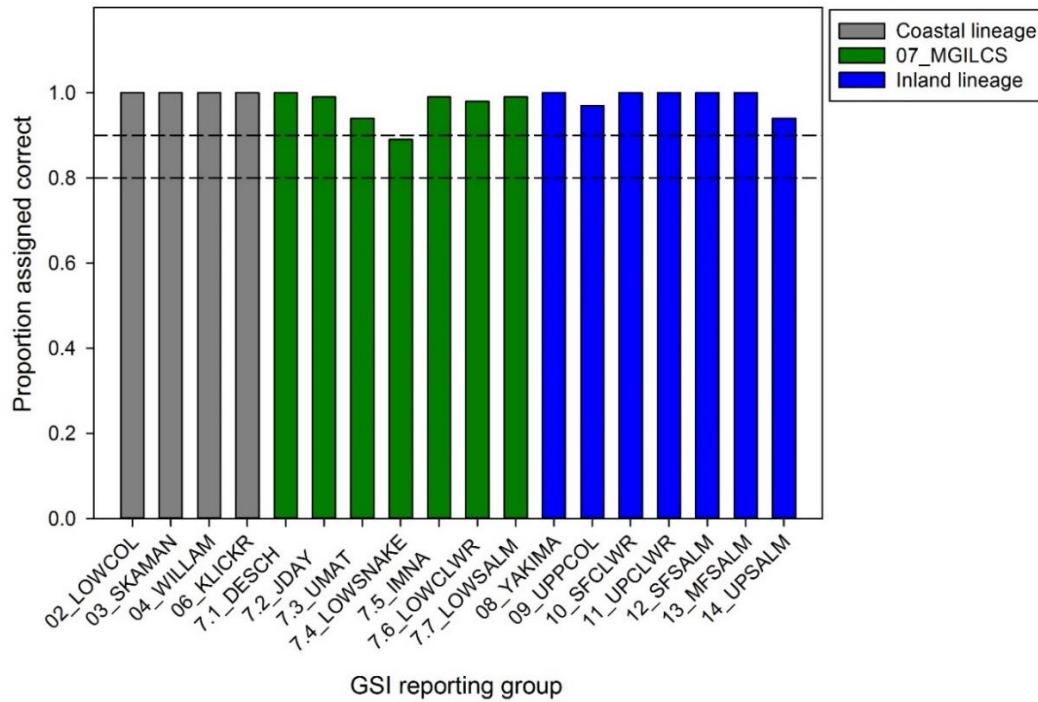


Figure 7: Reporting group assignment success with new 333 SNP GT-seq panel which incorporates the 63 MGILCS differentiating SNPS. Green bars indicate new reporting groups that fall within MGILCS. Dotted lines represent 80% and 90% correct assignment thresholds.

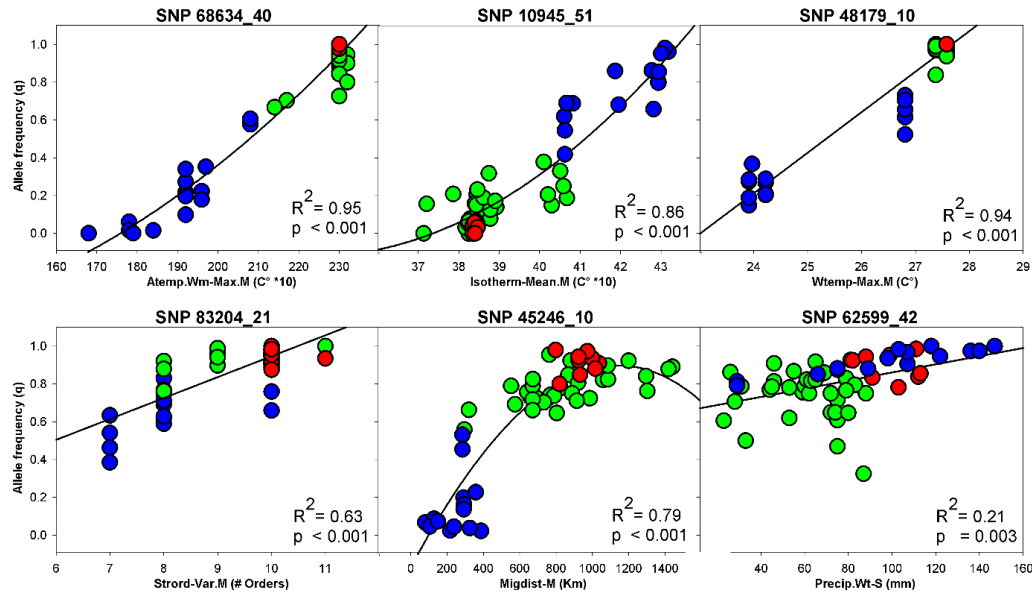


Figure 8: Example of strong correlations between certain environmental variables and allele frequencies of markers that have been incorporated into the GT-seq panel.

The GREB1 gene is located on chromosome 28 and was determined to be associated with run-timing based on FST and CMH analyses. Within this highly differentiated DNA region, a subset of 6 markers with the highest FST (> 0.8) had appropriate flanking sequence for primer design which were developed for incorporation into our existing SNP maker panel (**Table 3**).

Table 3: Markers developed for run timing using Pool-seq data from Klickitat and Kalama summer and winter populations.

Locus/SNP Name	Purpose	Gene
OMY_RADOMy-GREB1-03	Summer (premature) and Winter (mature) run timing	GREB-1L
OMY_RADOMy-GREB1-05	Summer (premature) and Winter (mature) run timing	GREB-1L
OMY_RADOMy-GREB1-06	Summer (premature) and Winter (mature) run timing	GREB-1L
OMY_RADOMy-GREB1-07	Summer (premature) and Winter (mature) run timing	GREB-1L
OMY_RADOMy-GREB1-09	Summer (premature) and Winter (mature) run timing	GREB-1L
OMY_RADOMy-GREB1-10	Summer (premature) and Winter (mature) run timing	GREB-1L

O. nerka

Estimates of pairwise variation (genetic distance) among 20 baseline populations ranged from a high of $F_{ST}=0.486$ between Redfish Lake Sockeye Salmon and Whatcom Lake kokanee, to a low of $F_{ST}=0.000$ between kokanee from Meadow Creek spawning channel and kokanee from Dworshak Reservoir ($P=0.4004$). All but the latter comparison indicated significant pairwise genetic variation ($P<0.0001$) between pairs of populations. The population with the highest mean pairwise F_{ST} in population pairwise comparisons was Redfish Lake Sockeye Salmon (0.394).

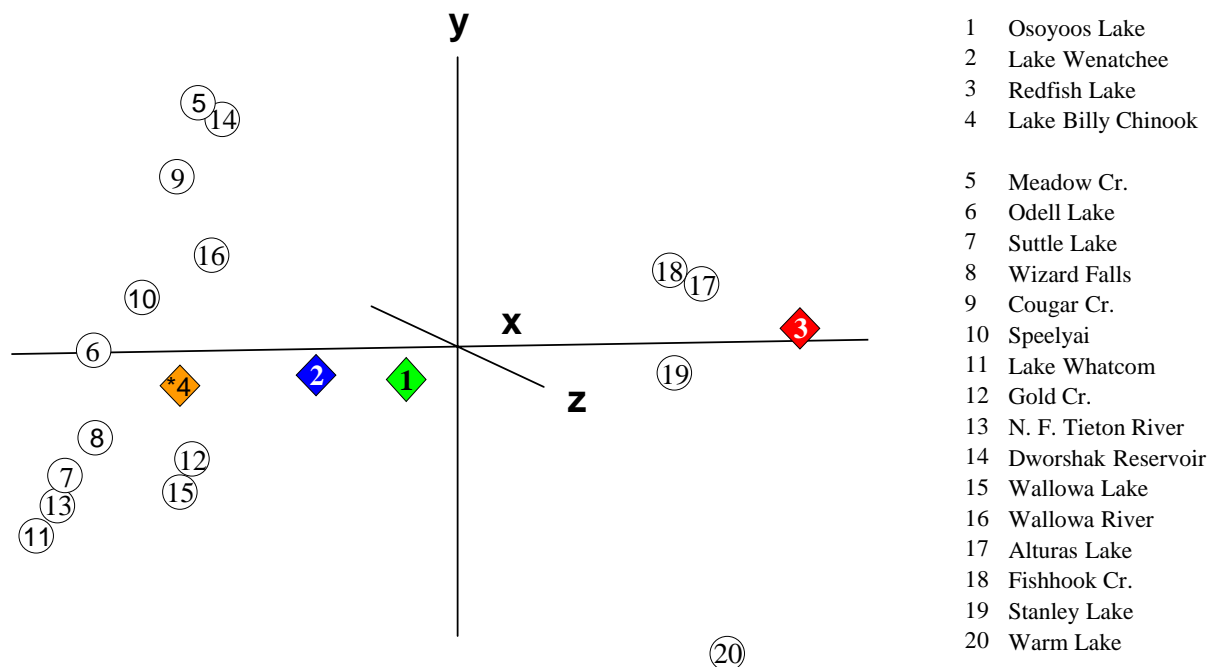


Figure 9: PCoA plot for *O. nerka*. Population clustering is based on standardized covariance of pairwise genetic distance. Sockeye Salmon populations distinguished by color are: red – Redfish Lake, orange – Lake Billy Chinook (*putative life history), green – Osoyoos Lake, and blue – Lake Wenatchee.

A principal coordinate analysis (PCoA) plot and the topology of an unrooted NJ phylogram (Figures 9 & 10) revealed defined clustering of the most genetically similar collections for *O. nerka*, including a broad distinction between Sockeye Salmon and kokanee populations.

Kokanee hatchery stocks from Meadow Creek and Whatcom Lake cluster among populations which have likely been stocked with outplants from either or both hatchery stocks in the past. Four kokanee populations from the Sawtooth Valley lakes region and from Warm Lake are highly distinct, and cluster more closely with Sockeye Salmon than with other kokanee populations in the baseline. These results corroborate the results of assignment tests indicating the accuracy of genetic stock identification to identify population of origin (i.e. 100% self-assignment; Table 1).

Discussion

Large datasets produced by genomic sequencing techniques may contain informative markers for population assignment. An original *O. mykiss* marker panel being used prior to 2017 could not effectively assign fish to basins within MGILCS; however, mining the RAD dataset from Micheletti *et al.* (2017) using population genetic tools identified a small set of 63 markers that increased assignment success while breaking MGILCS into 7 smaller reporting groups. Thus, the most recent *O. mykiss* marker panel, which incorporates these 63 markers, will provide higher resolution for genetic assignment and thus be more informative to managers. The ability to predict environmental conditions from genotyping adaptive markers can facilitate conservation of *O. mykiss* populations that will undoubtedly experience climatic variation. 63 adaptive markers illustrated very strong associations with 12 unique environmental variables and have been added to the *O. mykiss* marker panel. Genotyping and monitoring of fish on an annual basis will give insight to how environmental conditions are changing and how population allele frequencies are reacting. The addition of markers that can differentiate summer and winter-run *O. mykiss* will increase the confidence of determining an individual's run timing phenotype. In the end, this will lead to better estimation of the proportion of winter and summer-run fish in tributaries.

Over the course of the 10 year genetic assessment project we have compiled extensive data sets of SNP genotypes for Chinook Salmon steelhead trout and *O. nerka*, and to a lesser degree for Coho Salmon and Pacific Lamprey. The populations we have evaluated span diverse regions in the Col. River Basin (including the Snake River Basin). We continue to update and maintain SNP reference baselines for continued evaluation of these species in future generations. This longterm strategy informs harvest management and assures the greatest likelihood of discerning temporal variation among reproductively distinct species aggregates (Waples 1991), while monitoring population viability related to demographic trends that occur locally and/or regionally. Philopatry (Quinn *et al.* 1991, Hendry *et al.* 2003) and hatchery supplementation activities (Ford *et al.* 2006; Hard & Heard 1999) play a major role in how genetic divergence and differentiation is distributed geographically. For managing sustainable fisheries, it is necessary to understand the magnitude of influence that these and other factors have on our ability to differentiate populations, both qualitatively (phenotypes; landscapes) and quantitatively (e.g., genetic stock identification). This becomes particularly important where mixed stock fisheries may consist of both ESA listed and unlisted populations, and where differential harvest may have the greatest impact on specific populations. Our current efforts have largely focused on the discovery of outlier loci for adaptive divergence and on strengthening our understanding of non-neutral genetic variation among populations. Data collected through RAD sequencing and GT-seq techniques has yield tens of thousands of potential SNPs, and demonstrated their utility for characterizing adaptive variation, and identifying environmental and life history related variables that are likely to have significant influence on allele frequencies (e.g., precipitation, temperature, run-timing.; Hecht *et al.* 2015, Hess *et al.* 2016b; Micheletti *et al.* 2017). The expansion efforts reported here also provided improved ability to differentiate stocks on regional and local scales through application of GSI and PBT methods. The continued expansion of SNP panels and updating of baseline that include a more geographically broad set of collections/populations will help us achieve a greater level of resolution in such applications as GSI. This means greater statistical power to identify population distinctions among the major tributaries and subbasins of

the Columbia River Basin as we have demonstrated by refining a single large reporting group (MGILCS) into multiple smaller reporting groups for steelhead.

Collections of *O. tshawytscha*, *O. mykiss*, and *O. nerka* have been chosen for baseline expansion based on availability, novelty, and in accordance with our goal of reaching complete coverage of extant stocks within the Columbia River Basin. Priority collections for all three species have been identified as those important to basin-wide harvest and hatchery management, particularly in tribal fisheries. This includes major supplementation stocks for all three species: lower Columbia, ocean-type, and stream-type lineages of Chinook Salmon, inland and coastal lineages and summer-run and winter-run ecotypes of steelhead trout, and the anadromous (Sockeye Salmon) and land-locked (kokanee) forms of *O. nerka*. Species-specific reference baselines may include life history variants such as potentially distinct populations of resident *O. mykiss* (Narum et al. 2008a; Narum et al. 2011). The application of GSI in fisheries continues to inform managers on several fronts, including: harvest management, abundance estimates, life history distinctions and conservation needs. Moreover, PBT is being used for multiple purposes including validation of assigned origins using GSI. In fact, PBT frequently reveals substantial numbers of unmarked hatchery-origin fish that are incorrectly identified as wild in the field. Future efforts for baseline expansion include compiling marker “banks” that can be drawn from at any time should the need for more markers be necessary. An example of such need is basin-wide coverage to account for stock transfers or reintroductions throughout the basin (e.g., *O. nerka* in Cle Elum Lake).

References

- Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: a workbench to detect molecular adaptation based on a F_{ST}-outlier method. *BMC bioinformatics*, 9(1), 323.
- Ackerman, M., and M. Campbell. 2012. Chinook and steelhead genotyping for genetic stock identification at lower granite dam. ANNUAL PROGRESS REPORT July 1, 2011 - June 30, 2012, Submitted to Bonneville Power Administration. Contract # 53239; Project # 10-026-00. Available at: <https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P128035>
- Baird, N. A., P. d. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko and E. A. Johnson. 2008. Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers *PLoS ONE* 3(10): 7 pages.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.
- Beacham, T. D., K. L. Jensen, J. Supernal, M. Wetly, L. Deng and N. Varnavskaya. 2006. Pacific rim population structure of Chinook Salmon as determined from microsatellite analysis. *Transactions of the American Fisheries Society* 135:1604-1621.
- Blankenship, S. M., M. R. Campbell, J. E. Hess, M. A. Hess, T. W. Kassler, C. C. Kozfkay, A. P. Matala, S. R. Narum, M. M. Paquin, M. P. Small, J. J. Stephenson, K. I. Warheit and P. Moran. 2011. Major Lineages and Metapopulations in Col. River *Oncorhynchus mykiss* Are Structured by Dynamic Landscape Features and Environments. *T. Am. Fish. Soc.* 140:665–684.
- Bourret V, Kent MP, Primmer CR et al. (2013) SNP-array reveals genome-wide patterns of

- geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). *Molecular Ecology*, 22, 532–551.
- Byrne A., J. Hymer, S. Ellis, R. Dick II, K. Keller, C.A. Steele, J.E. Hess, M. Begay, and Miller T. 2015. A genetic analysis of the summer steelhead stock composition in the Col. River and Snake River tribal and sport fisheries. Idaho Department of Fish and Game, technical report number 15-06.
<https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res15-06Byrne13Genetic%Analysis%Summer%Steelhead%Stock%Col.-Snake%R.pdf>
- Campbell, N. R., and S. R. Narum. 2008. Identification of novel SNPs in Chinook Salmon and variation among life history types. *Transactions of the American Fisheries Society* 137:96-106.
- Campbell NR, Harmon SA, Narum SR. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* 15:855–867
- Catchen J. M., A. Amores, P. Hohenlohe, W. Cresko, J. H. Postlethwait. 2011. Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, 1, 171–182.
- Currens, K. P., C. B. Schreck and H. W. Li. 2009. Evolutionary ecology of redband trout. *Transactions of the American Fisheries Society* **138**:797–817.
- Dionne M., F. Caron, J. J. Dodson and L. Bernatchez. 2008. Landscape genetics and hierarchical genetic structure in Atlantic Salmon: the interaction of gene flow and local adaptation. *Molecular Ecology* **17**:2382-2396.
- Excoffier, L., G. Laval, and S. Schneider 2010. Arlequin ver. 3.5: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47–50.
- Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package), version 3.5c. Department of Genetics, University of Washington, Box 357360, Seattle, WA 98105, USA.
- Ford, M. J., H. Fuss, B. Boelts, E. LaHood, J. Hard and J. Miller. 2006. Changes in run timing and natural smolt production in a naturally spawning Coho Salmon (*Oncorhynchus kisutch*) population after 60 years of intensive hatchery supplementation. *Canadian Journal of Fisheries and Aquatic Sciences* 63:2343–2355.
- Fraser, D. J. and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology* **10**:2741-2752.
- Fraser, D. J., L. K. Weir, L. Bernatchez, M. M. Hansen and E. B. Taylor. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106**:404-4.
- Frichot, E., Schoville, S. D., Bouchard, G., & François, O. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular biology and evolution*, 30(7), 1687-1699.
- Funk, W. C., J. K. McKay, P. A. Hohenlohe and F. W. Allendorf. 2012. Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution* **27**(9):489-496.
- Galbreath, P.F., Bisbee, M.A., Dompier, D.W., Kamphaus, C.M., and Newsome, T.H. 2015. Extirpation and tribal reintroduction of coho salmon to the interior Columbia River basin. *Fisheries*, 39(February 2015): 77–87. doi:10.1080/03632415.2013. 874526.
- Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, 195(1), 205-220.
- Gustafson, R. G., T. C. Wainwright, G. A. Winans, F. W. Waknitz, L. T. Parker, and R. S.

- Waples. 1997. Status review of Sockeye Salmon from Washington and Oregon. U. S. Dept. Commer., NOAA Technical Memorandum MNFS-NWFSC-33, 282 p.
- Hard, J. J., and W. R. Heard. 1999. Analysis of straying variation in Alaskan hatchery Chinook Salmon (*Oncorhynchus tshawytscha*) following transplantation. *Canadian Journal of Fisheries and Aquatic Sciences* 56:578-589.
- Hasler, A. D., and A. T. Scholz. 1983. Olfactory imprinting and homing in salmon: investigations into the mechanism of imprinting process. *Zoophysiology*, Volume 14. Springer-Verlag, New York.
- Hasselman, Daniel J., Nathan R. Campbell, Andrew P. Matala, Stephen J. Micheletti, Stephanie A. Harmon, and Shawn R. Narum. 2016. GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS, 4/1/2015 - 3/31/2016 Annual Report, 2008-907-00
- Heard, W. R., R. Burkett, F. Thrower, and S. McGee. 1995. A review of Chinook Salmon resources in Southeast Alaska and development of an enhancement program designed for minimal hatchery-wild interaction. *American Fisheries Society Symposium* 15:21-37.
- Hecht BC, Campbell NR, Holecek DE, Narum SR. 2013. Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. *Molecular Ecology*, doi: 10.1111/mec.182.
- Hecht, B. C., A. P. Matala, J. E. Hess, and S. R. Narum. 2015. Environmental adaptation in Chinook Salmon (*Oncorhynchus tshawytscha*) throughout their North American range. *Molecular ecology*, 24(22), 5573-5595.
- Hendry, A. P., V. Castaic, M. T. Kinnison, and T. P. Quinn. 2003. The evolution of philopatry and dispersal: homing vs. straying in salmonids. In *Evolution illuminated: salmon and their relatives*. Edited by A. P. Hendry and S. C. Stearns. Oxford Univ. Press, New York, NY. pp. 52-91.
- Hess, J. E. and A. P. Matala. 2013. Archival genetic analysis suggests recent immigration has altered a population of Chinook Salmon in an unsupplemented wilderness area. *Conservation Genetics* DOI 10.1007/s10592-013-0546-z.
- Hess, J. E., N. R. Campbell, A. P. Matala and S. R. Narum. 2012. Genetic Assessment of Col. River Stocks. Col. River Inter-Tribal Fish Commission annual report 2011. Project Number: 08-907-00, Contract Number: 41224, Submitted to Bonneville Power Administration, March 31, 12.
- Hess J.E., N.R. Campbell, A.P. Matala, D.J. Hasselman, and S.R. Narum. 2015. 14 Annual Report: Genetic assessment of Columbia River stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #08-907-00.
<https://pisces.bpa.gov/release/documents/DocumentViewer.aspx?doc=P147368>
- Hess M.A., J.E. Hess, A.P. Matala, R.A. French, C.A. Steele, J. Lovtang, and S.R. Narum. 2016a. Migrating adult steelhead utilize a thermal refuge during summer periods with high water temperatures. *ICES Journal of Marine Science* 73(10):2616–2624
- Hess, J. E., Zendt, J. S., Matala, A. R., & Narum, S. R. (2016b). Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. In *Proc. R. Soc. B* (Vol. 283, No. 1830, p. 20153064). The Royal Society.
- Hess J.E., N.R. Campbell, A.P. Matala, D.J. Hasselman, and S.R. Narum. 2016c. Annual Report: Genetic assessment of Col. River stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #08-907-00.
<https://pisces.bpa.gov/release/documents/DocumentViewer.aspx?doc=P147368>

- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International journal of climatology*, 25, 1965-1978.
- Homer CG, Dewitz JA, Yang L, Jin S, Danielson P, Xian G, Coulston J, Herold ND, Wickham JD, Megown K (2015) Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*, 81, 345-354.
- Isaak, D. J., Wenger, S. J., Peterson, E. E., Ver Hoef, J. M., Nagel, D. E., Luce, C. H., ... & Chandler, G. L. (2017). The NorWeST Summer Stream Temperature Model and Scenarios for the Western US: A Crowd- Sourced Database and New Geospatial Tools Foster a User Community and Predict Broad Climate Warming of Rivers and Streams. *Water Resources Research*.
- Iwamoto EM, Myers JM, Gustafson RG. 2012. Resurrecting an extinct salmon evolutionarily significant unit: archived scales, historical DNA and implications for restoration. *Molecular Ecology* 21:1567–1582.
- Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403-1405.
- Jombart, T., & I. Ahmed,. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27(21), 3070-3071.
- Kearns, M., & Ron, D. (1999). Algorithmic stability and sanity-check bounds for leave-one-out cross-validation. *Neural computation*, 11(6), 1427-1453.
- Kofler, R., Orozco-terWengel, P., De Maio, N., Pandey, R. V., Nolte, V., Futschik, A., ... & Schlötterer, C. (2011). PoPoolation: a toolbox for population genetic analysis of next generation sequencing data from pooled individuals. *PloS one*, 6(1), e15925
- Kozfkay CC, Campbell MR, Heindel JA, Baker DJ, Kline P, Powell MS, Flagg T (2008) A genetic evaluation of relatedness for broodstock management of captive, endangered Snake River sockeye salmon, *Oncorhynchus nerka*. *Conservation Genetics* 9:1421–1430.
- Landguth, E.L. and N. Balkenhol. 2012. Relative sensitivity of neutral versus adaptive genetic data for assessing population differentiation. *Conservation Genetics* 13(5):1-6.
- Latch, E. K., W. I. Boarman, A. Walde and R. C. Fleischer. 2011. Fine-scale analysis reveals cryptic landscape genetic structure in desert tortoises. *PLoS ONE* 6(11):e27794.
- Limborg, M. T., S. M. Blankenship, S. F. Young, F. M. Utter, L. W. Seeb, M. H. H. Hansen, and J. E. Seeb. 2011. Signatures of natural selection among lineages and habitats in *Oncorhynchus mykiss*. *Ecology and Evolution*. doi:10.1002/(ISSN)45-7758
- Matala, A. P., J. E. Hess and S. R. Narum. 2011. Resolving adaptive and demographic divergence among Chinook Salmon populations in the Col. River basin. *T. Am. Fish. Soc.* 140:783–807.
- Matala AP, Young W, Vogel JL, Narum SR. 2012 Influences of hatchery supplementation, spawner distribution and habitat on genetic structure of Chinook Salmon (*Oncorhynchus tshawytscha*) in the South Fork Salmon River, ID. *North American Journal of Fisheries Management*, 32, 346–359.
- Matala, A. P., M. Ackerman, M. Campbell and S. R. Narum. 2014. Relative contributions of neutral and non-neutral genetic differentiation to inform conservation of steelhead trout across highly variable landscapes. *Evolutionary Applications* 7:682–701.
- Matala, A. P., Hatch, D. R., Everett, S., Ackerman, M. W., Bowersox, B., Campbell, M., & Narum, S. (2016). What goes up does not come down: The stock composition and demographic characteristics of upstream migrating steelhead differ from post-spawn

emigrating kelts. *ICES Journal of Marine Science* 73(10):2595–2605. doi:
10.10193/icesjms/fsw109

Matala A. P., B. Allen, S. Narum, and E. Harvey. 2017. Restricted gene flow between resident *Oncorhynchus mykiss* and an admixed population of anadromous steelhead. *Ecology and Evolution* 7:8349–8362. <https://doi.org/10.1002/ece3.3338>

McIssac, D. O. and T. P. Quinn. 1988. Evidence for a hereditary component in homing behavior of Chinook Salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 45:21-25.

Micheletti, S. J., Matala, A. R., Matala, A. P., & Narum, S. R. (2017). Landscape features along migratory routes influence adaptive genomic variation in anadromous steelhead (*Oncorhynchus mykiss*). *Molecular ecology*.

Micheletti, S.J., Narum, S.R. Submitted. Utility of pooled sequencing for association mapping in non-model organisms. *Molecular ecology resources*.

Micheletti, S.J. An R package for fast modification of Genepop format. *In prep*

Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko and E. A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Research* 17:240-248.

Milner, A. M. and R. G. Bailey. 1989. Salmonid colonization of new streams in Glacier Bay National Park, Alaska. *Aquaculture and Fisheries Management* :179-192.

Moen, T., B. Hayes, M. Baranski, P. R. Berg, S. Kjoeglum et al., 2008. A linkage map of the Atlantic Salmon (*Salmo salar*) based on EST-derived SNP markers. *BMC Genomics* 9: 223.

Moran, P., D. J. Teel, M. A. Banks, T. D. Beacham, M. R. Bellinger, S. M. Blankenship, J. R. Candy, J. C. Garza, J. E. Hess, S. R. Narum, L. W. Seeb, W. D. Templin, C. G. Wallace, and C. T. Smith. 2013. Divergent life-history races do not represent Chinook Salmon coast-wide: the importance of scale in Quaternary biogeography. *Canadian Journal of Fisheries and Aquatic Sciences* 70:415–435.

Narum, S. R., J. S. Zendt, D. Graves and W. R. Sharp. 2008a. Influence of landscape on resident and anadromous life history types of *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences* 65:1013-1023.

Narum, S. R., T. L. Schultz, D. M. Van Doornik and D. Teel. 2008b. Localized genetic structure persists in wild populations of Chinook Salmon in the John Day River despite gene flow from outside sources. *Transactions of the American Fisheries Society* 137:1650-1656.

Narum, S. R., N. R. Campbell, C. C. Kozfkay and K. A. Meyer. 2010a. Adaptation of redband trout in desert and montane environments. *Mol. Ecol.* doi: 10.1111/j.1365-294X.10.04839.x.

Narum, S. R., J. Hess and A. P. Matala. 2010b. Examining Genetic Lineages of Chinook Salmon in the Col. River Basin. *Transactions of the American Fisheries Society* 139:1465–1477.

Narum, S. R., J. S. Zendt, C. Frederiksen, N. Campbell, A. Matala, and W. Sharp. 2011. Candidate Genetic Markers Associated with Anadromy in *Oncorhynchus mykiss* of the Klickitat River. *T. Am. Fish. Soc.* 140(3):843-854.

Nei, M. 1972. Genetic distance between populations. *The American Naturalist* 106:283-292.

Neville, H., D. Isaak, R. Thurow, J. Dunham and B. Rieman. 2007. Microsatellite variation reveals weak genetic structure and retention of genetic variability in threatened Chinook Salmon (*Oncorhynchus tshawytscha*) within a Snake R. watershed. *Conservation Genetics* 8:133-147.

- Olsen, J. B., T. D. Beacham, M. Wetklo, L. W. Seeb, C. T. Smith, B. G. Flannery and J. K. Wenburg. 2010. The influence of hydrology and waterway distance on population structure of Chinook Salmon *Oncorhynchus tshawytscha* in a large river. *Journal of Fish Biology* **76**:1128-1148.
- Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, 288-295. Program note available from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1471-8286.05.01155.x>
- Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L., & Estoup, A. (2004). GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of heredity*, *95*(6), 536-539.
- Quinn, T. P. 1993. A review of homing and straying of wild and hatchery-produced salmon. *Fisheries Research* 18:29-44.
- Quinn, T. P., R. S. Nemeth and D. O. McIsaac. 1991. Homing and straying patterns of fall Chinook Salmon in the lower Col. R.. *Transactions of the American Fisheries Society* 1:150-156.
- Rannala, B. and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences USA* 94:9197-9221.
- Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals [mdash] mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, *15*(11), 749-763.
- Segelbacher, G., S. A. Cushman, B. K. Epperson, M.J. Fortin, O. Francois, O. J. Hardy, R. Holderegger, P. Taberlet, L. P. Waits and S. Manel. 2010. Applications of landscape genetics in conservation biology: concepts and challenges. *Conservation Genetics* **11**:375-385.
- Sepulveda-Villet, O. J. and C. A. Stepien. 2012. Waterscape genetics of the yellow perch (*Perca flavescens*): patterns across large connected ecosystems and isolated relict populations. *Molecular Ecology* (23):5795-5826.
- Steele C.A., E.C. Anderson, M.W. Ackerman, M.A. Hess, N.R. Campbell, S.R. Narum, and M.R. Campbell. 2013. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. *Canadian Journal of Fisheries and Aquatic Sciences*, 70: 1046–1054. <http://www.nrcresearchpress.com/doi/pdf/10.1139/cjfas-12-0451>
- Steele C.A., M.W. Ackerman, J. McCane, M.R. Campbell, M.A. Hess, N.R. Campbell, and S.R. Narum. 2015. Parentage based tagging of Snake River hatchery steelhead and Chinook Salmon. RME Technical Report to Bonneville Power Administration. Project 10-031-00. <https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res16-02Steele15%PBT%Snake%River%Hatchery%Steelhead%and%Chinook%Salmon.pdf>
- Sweet, D., Lorente, M., Valenzuela, A., Lorente, J., & Alvarez, J. C. (1996). Increasing DNA extraction yield from saliva stains with a modified Chelex method. *Forensic science international*, *83*(3), 167-177.
- Vu N.V., M.W. Ackerman, K.K. Wright, J. McCane, M.R. Campbell, J.E. Hess, and S.R. Narum. 15. 2014 Annual Report: Chinook and steelhead genotyping for genetic stock identification at Lower Granite Dam. U.S. Dept. of Energy Bonneville Power Administration Report Project # 10-026-00. <https://collaboration.idfg.idaho.gov/FisheriesTechnicalReports/Res15-02Vu14Chinook-Steelhead%Genotyping%for%GSI%at%LGR.pdf>

- 323 Waples, R. S. 1991. Pacific Salmon, *Oncorhynchus* spp., and the definition of “species”
324 under the Endangered Species Act. *Marine Fisheries Review* 53:11-22.
- 325 Waples, R. S., D. J. Teel, J. M. Myers, and A. R. Marshall. 2004. Life-history divergence
326 in Chinook Salmon: historical contingency and parallel evolution. *Evolution* 58:386-403.

327 Appendix 1: Fifty-six localities used in the RAD study to expand the GT-seq SNP panel for steelhead
 328 trout. Each locality is identified by the lineage (Lin), primary life stage of samples (Stage), number of
 329 individuals from the locality (N) and whether the locality was used to develop SNPs for adaptive
 330 divergence or to differentiate between groups in the MGILCS reporting group (SNP development).
 331

Name	Abbr.	Subbasin	Lin	Stage	Lat	Long	N	SNP Development
Abernathy	ABER	Elochoman	C	A	46.23	-123.15	21	Adaptive
Asotin	ASOT	Asotin	IN	J/A	46.32	-117.14	45	Adaptive, MGILCS
Bargamin	BARG	Salmon	IN	J	45.57	-115.19	24	Adaptive
Boulder	BOUL	Clearwater	IN	J	46.68	-114.74	47	Adaptive
Captain John	CAPJ	Snake	IN	J	46.15	-116.93	43	Adaptive, MGILCS
Catherine	CATH	G Ronde	IN	J	45.31	-117.87	36	Adaptive, MGILCS
Chamberlain	CHAM	Salmon	IN	J	45.45	-114.93	48	Adaptive
Chiwaukum	CHIW	Wenatchee	IN	J	47.69	-120.74	34	Adaptive
Crooked	CROO	Clearwater	IN	A	45.82	-115.53	45	Adaptive
Dworshak	DWOR	Clearwater	IN	A	46.5	-116.33	27	Adaptive, MGILCS
Eagle	EAGL	Willamette	C	J/A	45.35	-122.38	46	Adaptive
EF Hood	EFHJ	Hood	C	J/A	45.56	-121.59	46	Adaptive
EF Lewis	ELEW	Lewis	C	A	45.85	-122.78	38	Adaptive
East Moose	EMOO	Clearwater	IN	J	46.19	-114.9	40	Adaptive
E Potlatch	EPOT	Clearwater	IN	A	46.8	-116.42	34	Adaptive, MGILCS
Fifteen	FIFT	Fifteenmile	IN	J	45.51	-121.13	47	Adaptive, MGILCS
Fish/Lochsa	FISH	Clearwater	IN	A	46.33	-115.35	47	Adaptive
Parkdale	PAHH	Hood	C	J	45.52	-121.62	46	Adaptive
Cow	IMNA	Imnaha	IN	J	45.77	-116.75	14	Adaptive, MGILCS
John Day	JDMA	John Day	IN	J	44.41	-119.12	30	Adaptive, MGILCS
Joseph	JOSE	G Ronde	IN	A	46.03	-117.02	46	Adaptive, MGILCS
Kalama - S	KALS	Kalama	C	A	46.03	-122.87	46	Adaptive
Kalama - W	KALW	Kalama	C	A	46.03	-122.87	36	Adaptive
Klickitat - S	KLIS	Klickitat	C	A	45.72	-121.26	132	Adaptive
Klickitat - W	KLIW	Klickitat	C	A	45.72	-121.26	99	Adaptive
Lake	LAKE	Clearwater	IN	J	46.46	-115	42	Adaptive
L Clearwater	LCLW	Clearwater	IN	J	45.75	-114.78	47	Adaptive
Methow	LIBB	Methow	IN	J	48.23	-120.11	18	Adaptive
Lick Creek	LICK	Salmon	IN	J	45.06	-115.76	43	Adaptive
Loon	LOON	Salmon	IN	J	44.81	-114.81	45	Adaptive
LittleSheep	LSHE	Imnaha	IN	J	45.48	-116.93	37	Adaptive, MGILCS
Marsh	MARS	Salmon	IN	J	44.45	-115.23	45	Adaptive
U John Day	NFJD	John Day	IN	J	44.59	-118.51	46	Adaptive, MGILCS
Mill Creek	MILL	M. Col.	IN	J	45.61	-121.19	46	Adaptive, MGILCS
Minthorn	MINT	Umatilla	IN	J/A	45.67	-118.62	47	Adaptive, MGILCS
Mission	MISS	Clearwater	IN	J	46.37	-116.74	40	Adaptive, MGILCS
Naches	NACH	Yakima	IN	J	46.86	-121.05	46	Adaptive
M John Day	MFJD	John Day	IN	J	44.84	-118.48	47	Adaptive, MGILCS
NF Lewis	NLEW	Lewis	C	A	45.96	-122.56	40	Adaptive
Little Rock	NSAN	Willamette	C	J	44.75	-122.4	28	Adaptive
Pahsimeroi	PAHH	Salmon	IN	A	44.66	-114.03	29	Adaptive
Rock	ROCK	M Col.	IN	J	45.75	-120.44	40	Adaptive, MGILCS
Satus	SATU	Yakima	IN	J	46.2	-120.61	47	Adaptive
Sawtooth	SAWN	Salmon	IN	J/A	44.15	-114.88	41	Adaptive
S John Day	SFJD	John Day	IN	J	44.33	-119.57	80	Adaptive, MGILCS
Skamania	SKAM	Willamette	C	A	45.24	-122.28	47	Adaptive
S Santiam	SSAN	Willamette	C	J/A	44.42	-122.67	31	Adaptive
Tenmile	TENM	Clearwater	IN	J	45.81	-115.68	41	Adaptive
Tucannon	TUCN	Tucannon	IN	A	46.31	-117.66	46	Adaptive, MGILCS
G. Ronde	UGRT	G. Ronde	IN	J	45.73	-117.86	43	Adaptive, MGILCS
Umatilla	UMAT	Umatilla	IN	J	45.7	-118.4	46	Adaptive, MGILCS
Yankee F	WFYF	Salmon	IN	J	44.35	-114.73	46	Adaptive
Whitebird	WHIT	Salmon	IN	J	45.75	-116.32	44	Adaptive, MGILCS
WF Hood	WHOO	Hood	C	J	45.56	-121.69	45	Adaptive
Winthrop	WNFH	Methow	IN	J	48.48	-120.19	47	Adaptive
Willamette	WWIL	Willamette	C	J	44.75	-123.15	44	Adaptive

Appendix 2: Chinook Salmon hatchery broodstock sampled for PBT baselines (see Figure 4). Genetic lineage is lower Col. (LC), interior ocean-type (IOT), and interior stream-type (IST). Year refers to the first year of PBT sampling for each hatchery: na – not currently a PBT hatchery. Some 2016 PBT samples have been archived awaiting shipment to the Hagerman Genetics Laboratory as of the drafting of this report. The project collaborators at Idaho Department of Fish and Game (IDFG) were responsible for genotyping of Snake River hatcheries (see “completed” column).

Map ID	Spawning hatchery	Run type	Lineage	Region	Latitude	Longitude	Year	2016 genotyping	
								Sampled	Completed
1	Klaskanine (NF	fall (tule)	LC	Col.	46.090	-	na	na	na
1	Klaskanine (SF	fall (tule)	LC	Col.	46.090	-	na	na	na
2	Big Creek	fall (tule)	LC	Col.	46.147	-	201	2016	770
3	Cowlitz Salmon	fall (tule)	LC	Col.	46.511	-	201	archiv	0
3	Cowlitz Salmon	spring	LC	Col.	46.511	-	201	archiv	0
4	Toutle	fall (tule)	LC	Col.	46.375	-	201	archiv	0
5	Kalama Falls	fall (tule)	LC	Col.	46.017	-	201	archiv	0
5	Kalama Falls	spring	LC	Col.	46.017	-	201	archiv	0
6	Lewis River	spring	LC	Col.	45.937	-	201	archiv	0
7	Clackamas	spring	LC	Col.	45.296	-	na	na	na
8	Marion Forks	spring	LC	Col.	44.612	-	na	na	na
9	South Santiam	spring	LC	Col.	44.416	-	na	na	na
10	McKenzie	spring	LC	Col.	44.118	-	na	na	na
11	Willamette	spring	LC	Col.	43.745	-	na	na	na
12	Washougal	fall (tule)	LC	Col.	45.653	-	201	archiv	0
13	Bonneville, Tanner	fall (tule)	LC	Col.	45.633	-	na	na	na
14	Spring Creek NFH	fall (tule)	LC	Col.	45.728	-	201	2016	4940
15	Little White Salmon	fall	IOT	Col.	45.719	-	201	2016	7477
16	Umatilla	fall	IOT	Col.	45.913	-	201	2016	?
17	Prosser	fall	IOT	Col.	46.215	-	201	2016	310
18	Ringold Springs	fall	IOT	Col.	46.514	-	201	2016	1016
19	Priest Rapids	fall	IOT	Col.	46.647	-	201	2016	5064
20	Lyons Ferry	fall	IOT	Snak	46.598	-	201	2016	IDFG
21	Nez Perce Tribal	fall	IOT	Snak	46.520	-	201	2016	IDFG
22	Eastbank	summer	IOT	Col.	47.530	-	201	2015	na
23	Entiat NFH	summer	IOT	Col.	47.698	-	201	2016	301
24	Wells	summer	IOT	Col.	47.947	-	201	2016	755
25	Chief Joseph	summer	IOT	Col.	48.001	-	201	2016	1047
15	Little White Salmon	spring	IST	Col.	45.719	-	201	2016	755
16	Umatilla	spring	IST	Col.	45.913	-	201	2016	464
20	Lyons Ferry	spring	IST	Snak	46.598	-	200	2016	IDFG
21	Nez Perce Tribal	spring	IST	Snak	46.520	-	200	2016	IDFG
22	Eastbank	spring	IST	Col.	47.530	-	201	2015	na
25	Chief Joseph	spring	IST	Col.	48.001	-	201	2016	566
26	Carson NFH	spring	IST	Col.	45.868	-	201	2016	1036
27	Parkdale	spring	IST	Col.	45.525	-	201	2016	302
28	Klickitat	spring	IST	Col.	46.041	-	200	2016	484
29	Warm Springs NFH	spring	IST	Col.	44.861	-	201	2016	631
30	Round Butte	spring	IST	Col.	44.605	-	201	2016	328
31	Cle Elum SRF	spring	IST	Col.	47.187	-	201	2016	622
32	Leavenworth NFH	spring	IST	Col.	47.558	-	201	2016	1005
33	Methow	spring	IST	Col.	48.477	-	201	2016	117
34	Winthrop NFH	spring	IST	Col.	47.558	-	201	2016	386
35	Lookingglass	spring	IST	Snak	45.732	-	200	2016	IDFG
36	Dworshak NFH	spring	IST	Snak	46.504	-	200	2016	IDFG
37	Clearwater	spring	IST	Snak	46.504	-	200	2016	IDFG
38	Rapid River	spring/sum	IST	Snak	45.354	-	200	2016	IDFG
39	SF Salmon, McCall	spring/sum	IST	Snak	44.908	-	200	2016	IDFG
40	Pahsimeroi	spring/sum	IST	Snak	44.684	-	200	2016	IDFG
41	Sawtooth	spring/sum	IST	Snak	44.150	-	200	2016	IDFG

*Only 50% of broodstock were sampled in 2015

** No broodstock were sampled in 2014

Appendix 3: Steelhead trout hatchery broodstock sampled for PBT baselines (see Figure 5). Genetic lineage is coastal or inland. Year refers to the first year of PBT sampling for each hatchery; na – not currently a PBT hatchery. Some 2016 PBT samples have been archived awaiting shipment to the Hagerman Genetics Laboratory as of the drafting of this report. The project collaborators at Idaho Department of Fish and Game (IDFG) were responsible for genotyping of Snake River hatcheries (see “completed” column). All Coho broodstocks sampled for PBT broodstock were designated for release of fish upstream of Bonneville Dam.

Map ID	Spawning hatchery	Run type	Lineage	Region	Latitude	Longitude	Year	2016 genotyping	
								Sampled	Completed
<u>Steelhead trout</u>									
1	Big Creek	winter	coastal	Col.	46.147	-123.581	na	na	na
2	Abernathy FTC	winter	coastal	Col.	46.226	-123.153	2012	archived	0
3	Cowlitz Trout	winter	coastal	Col.	46.511	-122.629	na	na	na
4	Kalama Falls	winter	coastal	Col.	46.017	-122.733	na	na	na
5	Merwin	winter	coastal	Col.	45.954	-122.564	na	na	na
6	Clackamas	winter	coastal	Col.	45.296	-122.362	na	na	na
7	Eagle Creek NFH	winter	coastal	Col.	45.276	-122.202	na	na	na
8	Sandy	winter	coastal	Col.	45.407	-122.254	na	na	na
9	Skamania	summer/winter	coastal	Col.	45.652	-122.168	2013	2016	*180
10	Parkdale	winter	coastal	Col.	45.525	-121.622	2012	2016	42
11	Round Butte	summer	inland	Col.	44.605	-121.277	2013	2016	900
12	Umatilla	summer	inland	Col.	45.913	-119.552	2012	2016	83
13	Eastbank	summer	inland	Col.	47.530	-120.293	2012	<u>2015</u>	na
14	Wells	summer	inland	Col.	47.947	-119.871	2013	2016	258
15	Methow (Twisp)	summer	inland	Col.	48.477	-120.205	2013	2016	18
16	Winthrop NFH	summer	inland	Col.	48.477	-120.205	2012	2016	100
17	Lyons Ferry	summer	inland	Snake	46.598	-118.226	2009	2016	IDFG
18	Wallowa	summer	inland	Snake	45.418	-117.302	2009	2016	IDFG
19	Oxbow	summer	inland	Snake	44.971	-116.853	2008	2016	IDFG
20	Dworshak NFH	summer	inland	Snake	46.504	-116.328	2008	2016	IDFG
21	Pahsimeroi	summer	inland	Snake	44.684	-114.039	2008	2016	IDFG
22	Sawtooth	summer	inland	Snake	44.150	-114.883	2008	2016	IDFG
<u>Coho Salmon</u>									
1	Umatilla	na	na	Col.	45.913	-119.552	2012	archived	0
2	Prosser	na	na	Col.	46.215	-119.760	na	<u>na</u>	na
3	Leavenworth NFH	na	na	Col.	47.558	-120.674	2012	2016	?
4	Winthrop NFH	na	na	Col.	48.477	-120.205	2012	2016	?
5	Dworshak NFH	na	na	Snake	46.504	-116.328	2012	archived	0

**Only hatchery broodstock used for release of fish upstream of Bonneville Dam were genotyped for PBT in 2016.*

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Appendix 4: Checklist of PBT broodstock collections that comprise the PBT baselines for Chinook salmon and steelhead from 2008 through 2016. Species-specific collections code along with run type and genetic lineage are provided for both species.

Hatchery	Species	Code	Run type	Lineage	Year									
					2008	2009	2010	2011	2012	2013	2014	2015	2016	
Clearwater Fish Hatchery	Chinook	OtsCLWH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Clearwater Fish Hatchery - Powell Facility	Chinook	OtsPOWP	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Dworshak National Fish Hatchery	Chinook	OtsDWOR	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Catherine Creek	Chinook	OtsCTHW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Grande Ronde	Chinook	OtsGRUW	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Imnaha River	Chinook	OtsIMNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Lookingglass Creek	Chinook	OtsLOOK	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Lostine River	Chinook	OtsLSTW	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	Chinook	OtsLYON	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Lyons Ferry Fish Hatchery - Tucannon River	Chinook	OtsTUCW ^a	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	Chinook	OtsLYON_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X	X	
McCall Fish Hatchery - Johnson Creek	Chinook	OtsJHNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	X	
McCall Fish Hatchery - South Fork Salmon	Chinook	OtsMCCA	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Nez Perce Tribal Fish Hatchery (Fall)	Chinook	OtsNPFH_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X	X	
Nez Perce Tribal Fish Hatchery (Spring)	Chinook	OtsNPFH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Pahsimeroi Fish Hatchery	Chinook	OtsPAHH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Rapid River Fish Hatchery	Chinook	OtsRAPH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery	Chinook	OtsSAWT	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Big Creek Hatchery	Chinook	OtsBIG	Fall	Interior ocean type	*	*	*	*	*	*	*	X	X	
Carson National Fish Hatchery	Chinook	OtsCAR	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Chief Joseph Hatchery (Spring)	Chinook	OtsCJH_sp	Spring	Interior stream type	*	*	*	*	*	*	X	X	X	
Chief Joseph Hatchery (Summer/Fall)	Chinook	OtsCJH_sufa	Summer	Interior ocean type	*	*	*	*	*	X	X	X	X	
Cowlitz Salmon	Chinook	OtsCOW	Spring	Interior stream type	*	*	*	*	*	*	*	*	*	
Eastbank Fish Hatchery	Chinook	OtsEASTBK	Summer	Interior ocean type	*	*	*	*	X	*	*	*	*	
Entiat National Fish Hatchery	Chinook	OtsENFH	Summer	Interior ocean type	*	*	*	*	*	X	X	X	X	
Kalama Falls	Chinook	OtsKAL	Spring	Interior stream type	*	*	*	*	*	*	*	*	*	
Klickitat State Fish Hatchery	Chinook	OtsKH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X	
Leavenworth National Fish Hatchery	Chinook	OtsLNFH	Spring	Interior stream type	*	*	*	*	*	X	X	X	X	
Lewis River	Chinook	OtsLEW	Spring	Interior stream type	*	*	*	*	*	*	*	*	*	
Little White Salmon National Fish Hatchery (Fall)	Chinook	OtsLWS_sufa	Fall	Interior ocean type	*	*	*	*	*	X	X	X	X	
Little White Salmon National Fish Hatchery (Spring)	Chinook	OtsLWS_sp	Spring	Interior stream type	*	*	*	*	*	X	X	X	X	
Methow State Fish Hatchery	Chinook	OtsMETH	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Parkdale Fish Facility	Chinook	OtsPFF	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Priest Rapids Hatchery	Chinook	OtsPRH	Fall	Interior ocean type	*	*	*	*	X	X	X	X	X	
Round Butte Fish Hatchery	Chinook	OtsRB	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Ringold Springs State Hatchery	Chinook	OtsRGS	Fall	Interior ocean type	*	*	*	*	*	*	*	*	X	
Spring Creek NFH	Chinook	OtsSPCR	Fall	Interior ocean type	*	*	*	*	*	*	*	X	X	
Toutle	Chinook	OtsTOU	Fall	Interior ocean type	*	*	*	*	*	*	*	*	*	
Umatilla Fish Hatchery (Fall)	Chinook	OtsUMA_sufa ^b	Fall	Interior ocean type	*	*	*	*	X	X	X	X	X	
Umatilla Fish Hatchery (Spring)	Chinook	OtsUMA_sp	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Washougal	Chinook	OtsWAS	Fall	Interior ocean type	*	*	*	*	*	*	*	*	*	
Warm Springs National Fish Hatchery	Chinook	OtsWSNFH	Spring	Interior stream type	*	*	*	*	X	X	X	X	X	
Wells Fish Hatchery	Chinook	OtsWELLS	Summer	Interior ocean type	*	*	*	*	X	X	X	X	X	
Winthrop National Fish Hatchery	Chinook	OtsWTP	Spring	Interior stream type	*	*	*	*	*	X	X	X	X	
Yakima Nation Prosser Hatchery	Chinook	OtsPRO	Fall	Interior ocean type	*	*	*	*	X	X	*	X	X	
Yakima River Roza Dam-Integrated	Chinook	OtsYRint	Spring	Interior stream type	*	*	*	*	X	*	X	X	X	
Yakima River Roza Dam-Segregated	Chinook	OtsYRseg	Spring	Interior stream type	*	*	*	*	X	*	*	X	X	
Dworshak National Fish Hatchery	Steelhead	OmyDWOR	Unknown	Interior	X	X	X	X	X	X	X	X	X	
Little Sheep Creek Hatchery	Steelhead	OmyLSCR	Summer	Interior	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery- Touchet	Steelhead	OmyTOUW ^c	Summer	Interior	*	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	Steelhead	OmyLYON ^d	Unknown	Interior	*	X	X	X	X	N/A	N/A	N/A	N/A	
Lyons Ferry Fish Hatchery - Grande Ronde	Steelhead	OmyCGRW ^c	Summer	Interior	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery - Tucannon	Steelhead	OmyTUCW ^c	Summer	Interior	*	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery - Wallowa	Steelhead	OmyWALW	Summer	Interior	*	*	*	*	*	*	*	X	X	
Oxbow	Steelhead	OmyOXBO	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery	Steelhead	OmySAWT	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery - East Fork Salmon	Steelhead	OmyEFSW ^e	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery - Squaw Creek	Steelhead	OmySQUW ^f	Summer	Interior	X	X	X	X	X	X	X	X	X	
Pahsimeroi Fish Hatchery	Steelhead	OmyPAHH	Unknown	Interior	X	X	X	X	X	X	X	X	X	
Wallowa Fish Hatchery	Steelhead	OmyWALL	Summer	Interior	*	X	X	X	X	X	X	X	X	
Eastbank Hatchery	Steelhead	OmyEASTBK	Summer	Interior	*	*	*	*	X	X	X	X	*	
Methow Hatchery (Twisp)	Steelhead	OmyTWP	Summer	Interior	*	*	*	*	*	X	X	X	X	
Parkdale Fish Facility	Steelhead	OmyPFF	Winter	Coastal	*	*	*	*	X	X	X	X	X	
Round Butte Fish Hatchery	Steelhead	OmyRB	Summer	Interior	*	*	*	*	*	X	X	X	X	
Skamania Hatchery (Summer)	Steelhead	OmySKH_su ^g	Summer	Coastal	*	*	*	*	*	X	X	X	X	
Skamania Hatchery (Winter)	Steelhead	OmySKH_wi ^g	Winter	Coastal	*	*	*	*	*	X	X	X	X	
Umatilla Fish Hatchery	Steelhead	OmyUMA	Summer	Interior	*	*	*	*	X	X	X	X	X	
Wells Hatchery - Okanogan stock	Steelhead	OmyWEL	Summer	Interior	*	*	*	*	*	X	X	X	X	
Wells Hatchery - Omak stock	Steelhead	OmyWEL_OMA	Summer	Interior	*	*	*	*	*	*	*	X	X	
Winthrop National Fish Hatchery	Steelhead	OmyWTP	Summer	Interior	*	*	*	*	X	X	X	X	X	

- XChinook tissues genotyped using 298 SNPs
- XSteelhead tissues genotyped using 379 SNPs
- XSteelhead tissues genotyped using 269 SNPs
- XSteelhead tissues genotyped using 192 SNPs
- XChinook/Steelhead tissues genotyped using 96 SNPs
- ^aChinook Lyons Ferry stock consolidated under 'OtsLYON' starting in 2012
- ^bChinook Umatilla fall stock spawned at Little White Salmon Hatchery starting in 2015; not distinguished from LWS stock
- ^cSteelhead Lyons Ferry stock consolidated under 'OmyLYON' starting in 2012
- ^dSteelhead Lyons Ferry stock discontinued starting in 2013
- ^eSteelhead Sawtooth stock consolidated under 'OmySAWT' from 2012-2013
- ^fSteelhead Sawtooth stock consolidated under 'OmySAWT' in 2012; renamed 'Upper Salmon B-run' (YFLW) and consolidated under 'OmyPAHH' starting in 2013; spawned at Yankee Fork Weir by Shoshone-Bannock tribe beginning in 2014
- ^gSteelhead Skamania stock is collected late in calendar year, and is designated for the following broodyear (i.e., late 2012 collections are part of BY2013)
- N/A - Stock discontinued/non-existent
- *Broodstock not sampled

352 Appendix 5: Sixty-three environment-associated loci that have been incorporated into the marker panel.
353 For each locus, the ID, environmental association, and gene-BLAST match are present if applicable.

Locus/SNP_ID	Association Description	Blast Gene Hit
OMY_RAD103359-45	Max Water Temperature	unconventional myosin-If-like
OMY_RAD10733-10	Air and water temperature	None
OMY_RAD10945-51	Mean isothermality	None
OMY_RAD116-59	Max air temperature (driest quarter)	zinc finger 862-like
OMY_RAD12566-14	Max Water Temperature	synaptic vesicle membrane VAT-1 homolog
OMY_RAD13499-13	Natal Site Diurnal Range	None
OMY_RAD14033-46	Max air temperature (warmest quarter)	thyrotroph embryonic factor-like isoform X1
OMY_RAD15709-53	Natal site sothermality	None
OMY_RAD1751-18	Min isothermality	VHSV-induced protein-7 mRNA
OMY_RAD1919-22	Max Water Temperature	None
OMY_RAD19578-59	Natal Site precipitation (driest quarter)	None
OMY_RAD22123-69	Migration Distance to Ocean	MTSS1 isoform X1
OMY_RAD2277-7	Mean annual precipitation	lamin-L(III)-like isoform X2
OMY_RAD23354-66	Max air temperature (driest quarter)	lysM and peptidoglycan-binding domain
OMY_RAD24343-29	Mean air temperature (wettest quarter)	ras family member 11A-like
OMY_RAD25042-68	Max annual air temperature	oxysterol-binding -related 5-like
OMY_RAD25266-23	Natal site canopy cover	hemicentin-2 isoform X1
OMY_RAD26691-36	Max air temperature (warmest quarter)	Ankyrin repeat domain-containing 22
OMY_RAD27740-55	Mean wind velocity	None
OMY_RAD28236-38	Migration Distance to Ocean	guanine nucleotide-binding -like 3
OMY_RAD29559-69	Geological Breaks	fb growth factor 4A-like
OMY_RAD30230-25	Max air temperature (warmest quarter)	ADHE family 3 member B1-like
OMY_RAD30243-74	General topology (elevation and slope)	CUGBP Elav-like family member 3 isoform
OMY_RAD30392-17	Max air temperature (warmest quarter)	None
OMY_RAD31079-58	Max annual air temperature	solute carrier family 23 member 2-like
OMY_RAD33798-24	Min annual precipitation	polycomb SCMH1 isoform X2
OMY_RAD35005-13	Number of Damns passed	phosphatase 1L
OMY_RAD3651-48	Natal Site Diurnal Range	brain-specific angiogenesis inhibitor 1
OMY_RAD36952-53	Max air temperature (warmest quarter)	reticulon-4 receptor-like
OMY_RAD37492-53	Min isothermality	None
OMY_RAD38406-19	Max air temperature (warmest quarter)	None
OMY_RAD39156-33	Natal site air temperature (warmest quarter)	None
OMY_RAD3926-22	Migration Distance to Ocean	None
OMY_RAD40132-55	Natal site canopy cover	leucine-rich repeat neuronal 1-like
OMY_RAD40520-48	Natal Site annual precipitation	ATP-binding cassette B member 9
OMY_RAD40641-58	Max air temperature (warmest quarter)	tubby homolog isoform X1
OMY_RAD41594-34	Max air temperature (warmest quarter)	None
OMY_RAD42465-32	Max air temperature (warmest quarter)	regulation of nuclear pre-mRNA domain
OMY_RAD43117-55	Max Water Temperature	peptide-N asparagine amidase
OMY_RAD45246-10	Related to energy expenditure	serine threonine- kinase 35-like
OMY_RAD46452-51	Mean annual precipitation	quinone oxidoreductase
OMY_RAD4848-14	Air and water temperature	serine threonine- kinase 32C
OMY_RAD49637-74	Natal site isothermality	E3 ubiquitin- ligase RNF43
OMY_RAD5374-56	Min annual precipitation	zinc finger 385B isoform X1
OMY_RAD54441-29	Natal site slope	hepatocyte growth factor-like
OMY_RAD55404-54	Min annual precipitation	E3 ubiquitin- ligase DTX1 isoform X1
OMY_RAD55997-10	Range of heat load index	zinc finger OZF-like
OMY_RAD59758-41	Min annual precipitation	None
OMY_RAD60135-12	Migration Distance to Ocean	T-cell immunomodulatory
OMY_RAD65808-68	Mean precipitation (driest quarter)	growth arrest GADD45 beta-like
OMY_RAD66402-36	Natal Site annual precipitation	pigment epithelium-derived factor
OMY_RAD68634-40	Max air temperature (warmest quarter)	None
OMY_RAD72528-44	Range of annual air temperature	None
OMY_RAD76570-62	Min annual precipitation	coiled-coil domain-containing 137
OMY_RAD78147-27	Max air temperature (warmest quarter)	zinc finger MYM-type 1-like
OMY_RAD78502-57	Max air temperature (warmest quarter)	AP-3 complex subunit sigma-1 isoform X1
OMY_RAD78776-10	Max air temperature (warmest quarter)	regulation of nuclear pre-mRNA domain
OMY_RAD85131-35	Mean wind velocity	homeobox Nkx- -like
OMY_RAD86706-72	Mean slope	dynein regulatory complex 1-like
OMY_RAD9004-13	Mean air temperature (driest quarter)	heat shock factor 1-like isoform X1
OMY_RAD92485-64	Range of water temperature	zinc finger and BTB domain-containing 2
OMY_RAD9408-71	Number of stream orders crossed	None
OMY_RAD98715-53	Max air temperature (warmest quarter)	PHD finger 12

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Section 3: Genetic Stock Identification of Chinook Salmon, Sockeye Salmon, and Steelhead Harvest Mixtures in the Mainstem Columbia River

Introduction

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in mixed stock applications of salmonids (Shaklee et al. 1999, Winans et al. 2004, Beacham et al. 2006, and Beacham et al. 2011). These methods have been demonstrated to be useful even at relatively fine geographic scales within the Columbia River Basin (CRB) (Hess et al. 2011, Hess and Narum 2011, Hess et al. 2014). Within the CRB, Chinook salmon consist of three major genetic lineages and steelhead consist of two major genetic lineages that can be further divided into populations that are genetically structured on a finer spatial scale (e.g., Waples et al. 2004; Narum et al. 2010; Blankenship et al. 2011). In this study, we used separate groups of SNP markers to discriminate 19 reporting groups for Chinook salmon, 14 reporting groups for steelhead, and four reporting groups for sockeye salmon.

Despite continuous improvements of the power of our Chinook salmon and steelhead baselines in GSI applications (Hess et al. 2014), we have determined that further improvement in the detail of data and accuracy of stock assignments could be made by utilizing a recently developed genetic technology (i.e., parentage based tagging (PBT)), in combination with GSI, in a tiered approach for stock identification (Hess et al. 2016). PBT is an efficient approach for mass tagging of fish. The method is carried out by first genotyping a set of potential parents which then provides the opportunity to assign a set of genotyped offspring to their true parent pair. PBT is currently being utilized on a broad scale in the Columbia River Basin, and was recently (i.e., 2012-present) expanded beyond Snake River hatcheries (Steele et al. 2011) to tag all Chinook salmon and steelhead hatchery broodstock from hatcheries in the CRB above Bonneville Dam. This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years, and elsewhere in the CRB above Bonneville Dam beginning with the 2012 brood year. When parent pairs of hatchery fish are identified with PBT, we can provide accurate information including age of the fish and the source hatchery in which its parents were spawned (Steele et al. 2011). We use PBT in this harvest study to identify hatchery-origin fish, and then use GSI to estimate stock-of-origin of all other hatchery fish that were not assigned with PBT and for all natural origin fish. For sockeye salmon, we rely solely on GSI to determine stock of origin since PBT is not necessary to identify stocks. For the 2016 Chinook harvest, multiple age classes (3-, 4-, and 5-year old fish) can be identified from Snake River stocks using PBT. However, because our PBT baseline continues to expand for Columbia River stocks, primarily 3- and 4-year old fish can currently be identified for certain Columbia River stocks (Table 3).

We continue to employ the genotyping-in-thousands by sequencing (GT-seq) approach that has been developed in our laboratory (Campbell et al. 2015). This approach has increased

the cost-effectiveness for genotyping moderate numbers of SNP loci (100s) for relatively large numbers of individuals (1000s), which allows us to run all SNP loci regardless of whether we intend to use primarily PBT analyses or a combination of PBT and GSI. Thus, our projects now benefit from the additional data that comes from genotyping with all available markers (i.e., increased power for statistical assignment of individuals).

Fisheries conducted in the mainstem of the lower and middle Columbia River provide an important application of genetic stock analyses because the fish harvested consist of mixtures of stocks throughout the CRB. Further, mainstem Chinook salmon fisheries represent a majority of the CRB harvest of this species taken by the commercial, sport, and tribal fishermen. In order to help support sustainable fisheries, PBT and GSI can be used to address two primary questions: 1) how are Chinook salmon stocks temporally and spatially distributed in the mainstem Columbia River; and 2) how are these stocks temporally and spatially distributed in the harvests of fisheries.

Project objectives and higher-level harvest management questions

Our study had two primary objectives: 1) utilize a combination of PBT and GSI analyses to determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River, and 2) utilize GSI to estimate stock composition of sockeye salmon harvested above and below Bonneville Dam in commercial, sport, and tribal fisheries. Results from these objectives were used to address:

Harvest RM&E: F&W Program Management Question: What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?

Increasingly, we are tailoring our analyses to address specific questions that fisheries managers have presented to us. For example, in 2012 managers proposed extending the geographic boundary of one of the mark selective spring-run Chinook salmon sport fisheries above Bonneville Dam that occurs at the mouth of the Wind River. This extension created a larger “bubble” boundary at the mouth of the Wind River and was intended to increase Columbia River mainstem fishing access while maintaining targeted focus on Wind River spring-run Chinook salmon. For 2012-2015, we examined the stock composition of the Wind River sport harvest and provided context by comparing stock proportions among the various samples from other fisheries and Bonneville Dam that were analyzed that same year. We repeated this analysis for fish harvested in 2016. We include in this report the 4th year of analysis of sockeye salmon fisheries in the Columbia River mainstem. Differences in relative abundance of the three main stocks (Okanagan, Wenatchee, and Snake) present challenges to managing lower river harvest, because of the desire to harvest the highly abundant Okanagan stock around the much less abundant Snake River stock and moderately abundant Wenatchee River stock. Stock composition estimates are expected to help determine how harvest is impacting these various stocks.

Time line for completion of objectives

Objectives will be ongoing and PBT/GSI results updated each year for harvest analyses of salmonids throughout the accords-funding. As new genetic techniques are developed they will be applied to this project and results will be compared between years to determine the extent of improvements.

Our study was not designed to address the following question:

Harvest RM&E: F&W Program Management Question: Can selective fisheries targeting hatchery fish or healthy populations reduce impacts on ESA-listed populations?

Accuracy testing of PBT and GSI baselines

Prior to conducting analyses for fisheries harvest collections and mixture samples encountered at Bonneville Dam (Section 4), we assessed the accuracy of our PBT and GSI baselines in assigning Chinook salmon and steelhead to their hatchery brood or reporting group of origin (see Results section).

Methods

Methods for estimating stock composition are available at (<https://www.monitoringmethods.org/Protocol/Details/229>). The Monitoring Methods Protocol is entitled Snake River steelhead and Chinook salmon stock composition estimates (2010-026-00) v1.0.

Tissue collection of Chinook salmon and Sockeye salmon

Tissues were sampled from Chinook salmon in 2016 from a total of 9 different mixture sources: the spring-run seasons of the following fisheries: 1) lower river commercial, 2) lower river sport, 3) lower river test, and 4) Wind R. sport, the summer management period harvests of the following fisheries: 5) lower river commercial, 6) lower river sport, and 7) Zone 6 tribal summer, and the fall-run harvest from 8) the lower river commercial and 9) Zone 6 tribal fall fishery. Tissues are also collected from steelhead, Chinook salmon, and sockeye salmon at Bonneville Dam for stock ID (see Section 4). Drano Lake samples from the spring sport fishery were not provided to us in 2016. While fisheries generally harvest jack sized Chinook salmon at low rates and do not have specific harvest limits on jacks, jacks do comprise part of the harvest and may be sampled if encountered. Jacks are sampled at the Bonneville AFF trap in the proportion that they are encountered in the sampling. Sampling restrictions at the AFF can result in biases in the size of fish sampled compared to the run at large. Harvest tissues were collected in coordination with existing monitoring programs led by Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW) and the Yakama Nation. The spring management period Chinook salmon fisheries were sampled below Bonneville Dam in the sport and commercial fishery and sampled above Bonneville Dam in Zone 6 as part of the Wind River sport fishery (Figure 1; Table 1). The summer management period fisheries were sampled below Bonneville Dam in the sport and commercial fisheries, and above Bonneville Dam in Zone 6 in the tribal commercial fishery. Due to limited funds, we analyzed a subset of samples obtained from the spring and summer Chinook salmon sport and

commercial fisheries below Bonneville. For fisheries in which we had to subsample the harvest, we selected fish randomly and with a balanced design across spatial regions.

Stock proportions were calculated for some groupings within each fishery source, such that stock proportions could be compared across geographic regions as well as adipose-clipped versus non-adipose-clipped categories for particular fisheries. We use the following four main geographic regions (Figure 1): Region A corresponds to our grouping of pre-existing Oregon and Washington state sport fishing zones 1-4 (or commercial zones 4-5), Region B corresponds to our grouping of sport zones 5-10 (or commercial zones 1-3). Here, we do not discriminate between Region 01 and Region 02 in the Zone 6 fishery, because that information did not accompany the samples we received. These sets of groupings were established for this study in order to achieve balanced sampling for analysis of these fishery datasets, as well as to set an appropriate spatial scale of analysis to minimize variance of our estimates of stock proportions over temporal strata.

Non-tribal fisheries during the spring management period for Chinook salmon are mark-selective based on absence or presence of the adipose fin to distinguish hatchery fish from natural origin fish, respectively. These adipose markings make it possible to have a mark-selective sport and commercial fishery in which only fish with missing adipose fins (hatchery-origin) are legally retained. Fish with intact adipose fins that are caught in these fisheries are released, but mortality rates are unknown from these releases. In addition to sampling hatchery-origin fish from the mark selective commercial and sport fisheries, we were able to obtain samples from non-clipped hatchery and natural origin fish from Bonneville Dam and the tribal Zone 6 fishery above Bonneville Dam.

Tissues were sampled from sockeye salmon in 2016 from four fishery mixture sources: 1) lower river commercial, 2) lower river sport, 3) Bonneville Dam (see Section 4), and 4) the tribal fishery in Zone 6. All samples obtained from these fisheries were analyzed.

Molecular data

Methods for DNA extraction, DNA amplification, and genotyping of SNP assays using genotyping-in-thousands by sequencing (GT-seq) are available at (<https://www.monitoringresources.org/Document/Method/Details/5446>). Additional details regarding how 192 SNPs were reduced to 186 SNPs can be found in Hess et al. (2012, 2013). Subsequently, we have reduced our Chinook salmon GSI baseline from 186 SNPs to 179 SNPs, because we were unable to transition the full set of 186 SNPs to GT-seq protocols. These 179 SNP markers were used for GSI, and for PBT analyses, we used 95 of the SNPs. We used 93 SNP markers for GSI of sockeye mixtures.

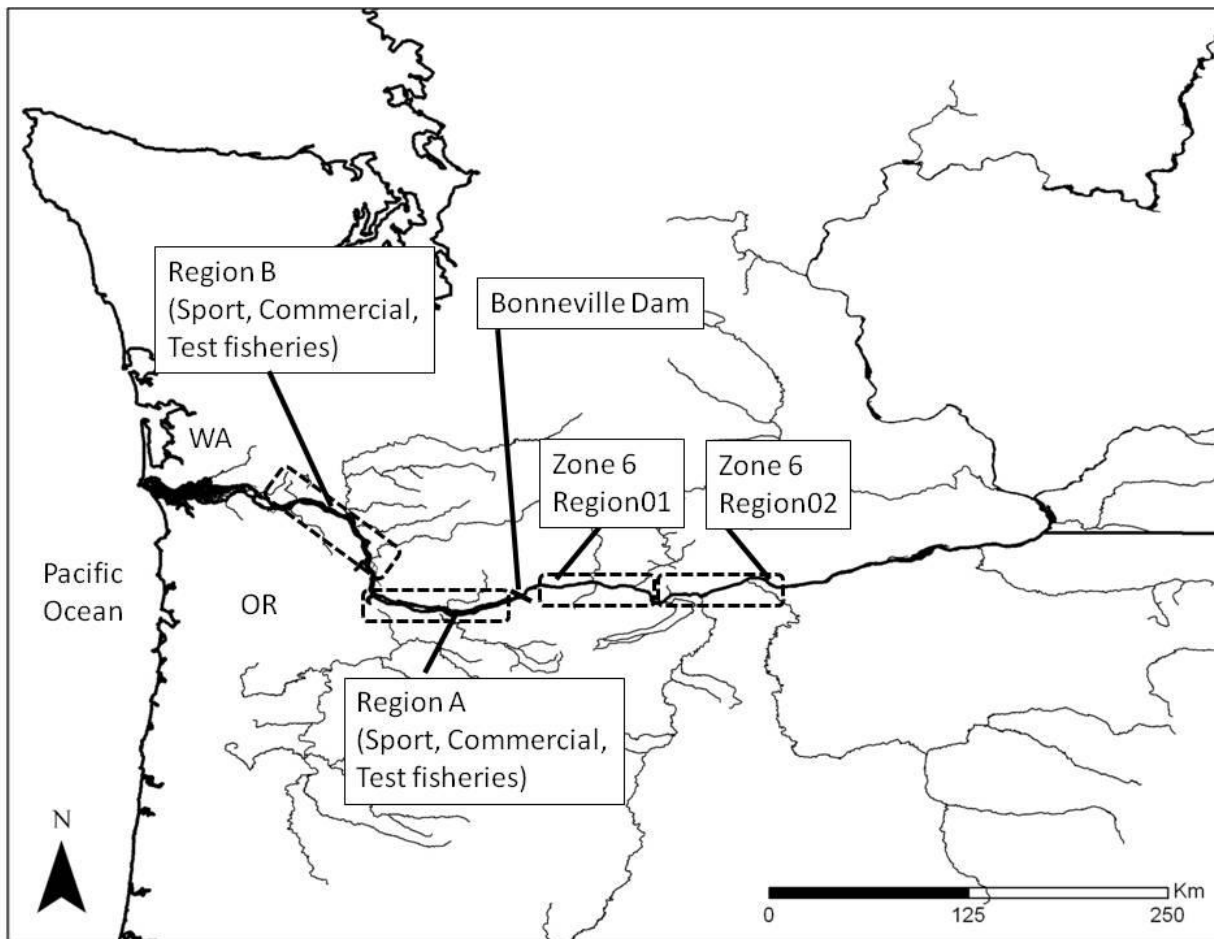


Figure 1: Project scope showing sources of Chinook salmon and sockeye salmon harvest mixtures that were analyzed using PBT/GSI.

522 Table 1: Characteristics of Chinook salmon harvest samples by fishery, region, life history stage, and origin by weekly strata in 2016.

Run Type	Region	Harvest Region	Stage	Origin	Sampled (N)	Genotyped (N)	Statistical week																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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Spring	Lower River Commercial	Region A	Adult	Hatchery	431	423						61	28					87		38	167	42																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
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GSI baselines for Chinook salmon, sockeye salmon, and steelhead

Chinook salmon GSI analyses were performed using the updated baseline referred to as “Columbia River Basin Chinook salmon GSI baseline version 3.1” and is available on the FishGen website (<https://www.fishgen.net>). This baseline consists of 61 collections that are delineated into the following 19 reporting groups: Columbia Rogue “01_YOUNGS”, West Cascade spring-run “02_WCASSP”, West Cascade fall-run “03_WCASFA”, Willamette River spring-run “04_WILLAM”, Spring Creek Group Tule fall-run “05_SPCRTU”, Klickitat River spring-run “06_KLICKR”, Deschutes River spring-run “07_DESCSP”, John Day River spring-run “08_JOHNDR”, Yakima River spring-run “09_YAKIMA”, upper Columbia River spring-run “10_UCOLSP”, Tucannon River spring-run “11_TUCANO”, Hells Canyon spring-run “12_HELLSC”, South Fork Salmon River spring-run “13_SFSALM”, Chamberlain Creek spring-run “14_CHMBLN”, Middle Fork Salmon River spring-run “15_MFSALM”, upper Salmon River spring-run “16_UPSALM”, Deschutes River fall-run “17_DESCFA”, upper Columbia River summer-/fall-run “18_UCOLSF”, and Snake River fall-run “19_SRFALL” (Table 2; Figure 2). Reporting groups were primarily determined by the relative genetic similarity among populations according to a phylogenetic analysis, and our previous results demonstrate sufficient power to discern three reporting groups (17_DESCFA, 18_UCOLSF, and 19_SRFALL) among the interior ocean-type collections. In one year, we had grouped all interior ocean-type collections into a single reporting group “Interior_Columbia_R_su/fa” (Hess et al. 2013). Genetic distances were computed from allele frequencies based on Nei’s (1972) genetic distance, with the PHYLIP v 3.69 (Felsenstein 1989) and 1000 bootstrap replicates were performed. Distances were clustered using the Neighbor – Joining method (Saitou and Nei, 1987), and a consensus tree was constructed (<http://evolution.genetics.washington.edu/phylip/>) (Figure 3).

The 10_UCOLSP reporting group includes the following Bonneville pool hatchery stocks: Carson stock (Ots22), and Little White Salmon R. (Ots23) because they are genetically indistinguishable from Upper Columbia R. spring Chinook salmon (includes Walla Walla and Umatilla River stocks). This composite group is notable because inclusion of these Bonneville pool stocks explains why a large proportion of fish from the Wind R. sport fishery should assign to this 10_UCOLSP reporting group. However, in future years the PBT baseline will be able to specifically identify fish from Carson Hatchery and Little White Salmon Hatchery allowing them to be distinguished from fish returning to the upper Columbia R. The 01_YOUNGS reporting group represents an out-of-basin genetic stock (originating from the Rogue R., OR) that is reared within the Columbia R. at Youngs Bay. Basic QAQC was performed to remove duplicate individuals and strays from the reference populations in the baseline. The baseline and reporting group data is available on FishGen.

GSI analyses for *O. nerka* utilized a baseline that included sockeye salmon and kokanee populations from throughout the Columbia River basin. This baseline included sockeye populations from the Okanogan, Wenatchee, and Redfish Lake, and a kokanee population from Lake Whatcom that were included in “Sockeye GSI baseline v1.0, and were shown to accurately discriminate among these major stock (Hess et al 2013). We updated our baseline to included additional sockeye and kokanee populations from Alturas Lake, Petit Lake, Fishhook Creek, Lake Billy Chinook, Meadow Creek, Suttle Creek, Upper Deschutes River, and Wallowa Lake,

and refer to this as “Sockeye GSI baseline v2.0”. The transition to GT-seq required omission of a few loci due to poor genotyping quality with the new protocols. A total of 90 SNPs was used for these analyses, however Section 2 provides a summary of a recently updated sockeye GSI baseline (382 SNPs) that will be applied for future stock ID analyses.

For steelhead, we used GSI baseline version 3.3 that comprises 116 collections from throughout the Columbia River basin that are partitioned into the following 14 reporting groups: 01_WCOAST (Quinalt River), 02_LOWCOL (lower Columbia River), 03_SKAMAN (Skamania hatchery releases at three sites in lower Columbia River, Willamette River, and Klickitat River), 04_WILLAM (Willamette River), 05_BWSALM (Big White Salmon River), 06_KLICKR (Klickitat River), 07_MGILCS (middle Columbia River, Grande Ronde River, Imnaha River, lower Snake River, lower Clearwater River, and lower Salmon River), 08_YAKIMA (Yakima River), 09_UPPCOL (upper Columbia River), 10_SFCLWR (South Fork Clearwater River), 11_UPCLWR (upper Clearwater River), 12_SFSALM (South Fork Salmon River), 13_MFSALM (Middle Fork Salmon River), and 14_UPSALM (upper Salmon River) (see Table 10; Figure 4). Genetic distances were computed from allele frequencies based on Nei’s (1972) genetic distance, with the PHYLIP v 3.69 (Felsenstein 1989) and 1000 bootstrap replicates were performed. Distances were clustered using the Neighbor – Joining method (Saitou and Nei, 1987), and a consensus tree was constructed (<http://evolution.genetics.washington.edu/phylip/>) (Figure 5).

Combined application of PBT and GSI

We combined PBT and GSI results together by first accepting all confident PBT assignments to hatchery broodstock for interior stream-type (i.e., $\text{LOD} \geq 14$ & $\text{FDR} \leq 0.1$) and interior ocean-type (i.e., $\text{LOD} \geq 14$ & $\text{FDR} \leq 3.0$) Chinook salmon (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341). For the remaining individuals, we used the best estimate of GSI assignments (probability of assignment ≥ 0.80) provided by the program ONCOR to determine likely reporting group of origin (Method: [Assigning individual samples using Individual Assignment \(IA\) genetic methods v1.0](#), ID: 1334). For the assignment of sockeye, GSI via ONCOR was used. For Chinook salmon, all age classes (3-, 4-, and 5-year old fish) can be identified from Snake River stocks using PBT. However, because our PBT baseline continues to expand for Columbia River stocks, only 3- and 4-year old fish collected in 2016 were identified for certain Columbia River stocks. For fish collected in 2017 and beyond, we will be able to identify 3, 4, and 5 year old age classes throughout most of the Columbia Basin since PBT baselines were initiated in 2012 outside of the Snake Basin.

Results

We first present results from our assessment of PBT and GSI baseline accuracy, and then address results from the assignment of the 2016 harvest samples.

Accuracy testing of the PBT baseline

Chinook (94 SNPs)

To test the accuracy of the Chinook salmon PBT baseline (94 SNPs) in assigning known samples to their hatchery brood of origin, we selected six hatchery collections from 2016 and one hatchery collection from 2015, and converted them to unknown samples (Table 4). These hatchery collections represented five interior stream type lineages (i.e., Klickitat Hatchery, McCall Hatchery (South Fork Salmon stock and Johnson Creek stocks), Sawtooth Fish Hatchery, and Round Butte Hatchery) and two interior ocean type lineages (i.e., Lyons Ferry Hatchery and Nez Perce Tribal Hatchery). These collections were chosen because of the availability of PBT baselines in previous years that these samples could be assigned to parents (Table 3). For the interior stream type lineage from the Klickitat Fish Hatchery, Sawtooth Hatchery and the McCall Fish Hatchery (South Fork Salmon and Johnson Creek stocks), we have PBT baselines that extend as far back as 2008 that allow us to assign fish from age classes 3, 4 and 5. However, for the interior stream type lineage from the Round Butte Fish Hatchery, our baselines extend to 2012 and only permits the assignment of age classes 3 and 4. For the interior ocean type lineage from the Lyons Ferry Fish Hatchery and Nez Perce Tribal Fish Hatchery, our baselines extend to 2011 and permit the assignment of fish from age classes 3, 4 and 5. After converting hatchery collections at each of these facilities to unknown samples, we conducted PBT assignments using brood years 2011-2015 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341). The expectation was that hatchery origin fish returning to each facility should assign to parent broodstock in the previous generation from the same hatchery. As described above, not all age classes were represented in PBT baselines for each program so we expected missing assignments for certain stocks. Even when all age classes are available, tagging rates may be lower than 100% that would also result in unassigned offspring. Further, some hatchery programs (e.g., McCall Hatchery Johnson Creek stock) integrate natural origin fish, so these fish would also not be expected to assign to parent broodstock in the previous generation. Given that some offspring did not have true parents in the PBT baseline, this approach allowed us to test for false positive assignments in addition to broodstock assignment accuracy. For the interior stream type collections, we used a LOD-score ≥ 14 and a False Discovery Rate (FDR) ≤ 0.6 to identify fish that were successfully ‘assigned’; whereas for interior ocean type collections we used a LOD-score ≥ 14 and a False Discovery Rate (FDR) ≤ 3.0 . All other fish that did not meet the necessary assignment criteria were considered ‘unassigned’.

645 *Round Butte Hatchery:*

646 Since the PBT baseline for the Round Butte hatchery only extends to BY2012 and
647 offspring from 2016 were tested, we only expected to assign 3- and 4-year old fish to parents. Of
648 the offspring tested, 80% of the Round Butte samples from 2016 were successfully assigned. Of
649 those fish that were assigned, 94% of the Round Butte fish assigned to their expected broodstock
650 (i.e. OtsRB12, OtsRB13, OtsWSNFH12, and OtsWSNFH13). Unexpected broodstock origins
651 for Round Butte included assignments to multiple hatcheries from the Snake River (Table 4).
652 Chinook salmon from the Snake River are known to stray into the Deschutes River, and it is
653 likely that some of these strays have been incorporated into the Round Butte Hatchery program
654 (i.e., these are not mis-assignments). This will be tested further with additional years of returns,
655 but initial data suggests that there were little to no false positive assignments.

656 *Klickitat Hatchery, McCall Hatchery and Sawtooth Hatchery:*

657 The Sawtooth and McCall (South Fork Salmon stock) samples from 2016 had the
658 greatest percentage of successful assignments (i.e., 95% and 94%, respectively), and reflects
659 more complete parental baselines available for assignment of all the age classes that are
660 anticipated to be encountered in these samples. Although only 59% of Klickitat samples were
661 successfully assigned, this is likely due to incomplete broodstock sampling (lower tagging rates)
662 and natural origin fish in the samples from 2016. Natural-origin fish were used almost
663 exclusively for the propagation of the Johnson Creek stock, and explains why only 4% of the
664 samples from 2015 were successfully assigned. Of the offspring that were successfully assigned,
665 nearly 100% of the Sawtooth, McCall (South Fork Salmon stock), and Klickitat samples were
666 assigned to their expected broodstock (Table 4). Unexpected broodstock origins for the
667 Sawtooth, McCall (South Fork Salmon stock) and Klickitat samples from 2016 included other
668 Snake River Hatcheries, and likely represent a small number of strays.

669 *Lyons Ferry Hatchery and Nez Perce Tribal Hatchery:*

670 For interior ocean type lineages (fall Chinook), 60% of the Lyons Ferry samples and 63% of the
671 Nez Perce samples from 2016 were successfully assigned (Table 4). This comparatively low
672 level of successful assignment may be attributable to the integration of natural origin fish in
673 these programs which would not be expected to assign to parent broodstock in the previous
674 generation. Of those fish that were successfully assigned, 51% of Lyons Ferry 2016 and 45% of
675 Nez Perce 2016 fish assigned to their expected broodstock (Table 4). Unexpected broodstock
676 origins for Lyons Ferry 2016 samples were predominantly from the Nez Perce hatchery program
677 (n=557/568; 98%), while unexpected broodstock origins for Nez Perce 2016 samples were
678 predominantly from the Lyons Ferry hatchery program (n=251/261; 96%), and likely reflect the
679 common source of broodstock used by these programs. Additional sources of unexpected
680 broodstocks for the Lyons Ferry 2016 and Nez Perce 2016 samples include Little White Salmon
681 (2013, 2014), Priest Rapids (2012, 2014) and Umatilla (2012, 2013) hatchery programs (Table
682 4), and likely represent stray fish.

683 Table 2: Sample sizes and reporting groups of Chinook salmon baseline populations. Lineages: ST (stream type), OT (ocean type), LC (Lower
684 Columbia).

ID	Collection	(n)	Lineage	Reporting Groups	Reporting Group description
OTS01	Youngs Bay fall-run	91	Rogue	01_YOUNGS	Youngs Bay- Columbia Rogue stock
OTS02	Cowlitz R spring-run	90	LC	02_WCASSP	West Cascade spring-run
OTS03	Kalama R spring-run	83	LC	02_WCASSP	West Cascade spring-run
OTS04	Cowlitz R fall-run	82	LC	03_WCASFA	West Cascade fall-run
OTS05	Elochoman R fall-run	86	LC	03_WCASFA	West Cascade fall-run
OTS06	Lewis R fall-run	93	LC	03_WCASFA	West Cascade fall-run
OTS07	NF Lewis fall-run	178	LC	03_WCASFA	West Cascade fall-run
OTS08	Sandy R fall-run	83	LC	03_WCASFA	West Cascade fall-run
OTS09	McKenzie R spring-run	78	LC	04_WILLAM	Willamette River spring-run
OTS10	N Santiam R spring-run	79	LC	04_WILLAM	Willamette River spring-run
OTS11	Sandy R spring-run	48	LC	04_WILLAM	Willamette River spring-run
OTS12	White Salmon fall-run	77	LC	05_SPCRTU	Spring Creek tule fall-run
OTS13	Spring Creek NFH tule fall-run	49	LC	05_SPCRTU	Spring Creek tule fall-run
OTS14	Klickitat R spring-run	84	ST	06_KLICKR	Klickitat River spring-run
OTS15	Shitike R spring-run	93	ST	07_DESCSP	Deschutes River spring-run
OTS16	Warm Springs R spring-run	90	ST	07_DESCSP	Deschutes River spring-run
OTS17	John Day R spring-run	78	ST	08_JOHNDR	John Day River spring-run
OTS18	Middle Fork John Day R spring-run	47	ST	08_JOHNDR	John Day River spring-run
OTS19	North Fork John Day R spring-run	42	ST	08_JOHNDR	John Day River spring-run
OTS20	American R spring-run	76	ST	09_YAKIMA	Yakima River spring-run
OTS21	Cle-Elum spring-run	88	ST	09_YAKIMA	Yakima River spring-run
OTS22	Winthrop NFH spring-run	82	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS23	little White Salmon R spring-run	93	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS24	Wenatchee R spring-run	109	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS25	Entiat R spring-run	98	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS26	Tucannon R spring-run	81	ST	11_TUCANO	Tucannon River spring-run
OTS27	Wenaha R spring-run	179	ST	12_HELLSC	Hells Canyon spring-run
OTS28	Lostine R spring-run	212	ST	12_HELLSC	Hells Canyon spring-run
OTS29	Grande Ronde R spring-run	314	ST	12_HELLSC	Hells Canyon spring-run
OTS30	Imnaha R spring-run	96	ST	12_HELLSC	Hells Canyon spring-run
OTS31	Lolo Cr spring-run	89	ST	12_HELLSC	Hells Canyon spring-run
OTS32	Red R spring-run	221	ST	12_HELLSC	Hells Canyon spring-run
OTS33	Powell R spring-run	56	ST	12_HELLSC	Hells Canyon spring-run
OTS34	Red R weir spring-run	91	ST	12_HELLSC	Hells Canyon spring-run
OTS35	South Forth Salmon R spring-run	139	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS36	Johnson Cr spring-run	137	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS37	Secesh R spring-run	252	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS38	Chamberlain Cr spring-run	219	ST	14_CHMBLN	Chamberlain Creek spring/summer-run
OTS39	Big Cr spring-run	139	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS40	Camas Cr spring-run	55	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS41	Loon Cr spring-run	107	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS42	Sulphur Cr spring-run	94	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS43	Bear Valley Cr spring-run	135	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS44	Capehorn Cr spring-run	214	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS45	Marsh Cr spring-run	228	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS46	North Fork Salmon R spring-run	55	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS47	Lemhi R spring-run	96	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS48	Pahsimeroi R spring-run	92	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS49	East Fork Salmon R spring-run	286	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS50	Salmon R spring-run	83	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS51	West Fork Yankee Fork spring-run	75	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS52	Valley Cr spring-run	100	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS53	Sawtooth Hatchery weir spring-run	186	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS54	upper Deschutes R fall-run	252	OT	17_DESCFA	Deschutes River fall-run
OTS55	lower Yakima R fall-run	62	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS56	Hanford Reach fall-run	93	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS57	Wenatchee R summer-run	92	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS58	Entiat R summer-run	51	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS59	Methow R summer-run	87	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS60	Lyons Ferry weir fall-run	90	OT	19_SRFALL	Snake River fall-run
OTS61	Clearwater R fall-run	228	OT	19_SRFALL	Snake River fall-run

685

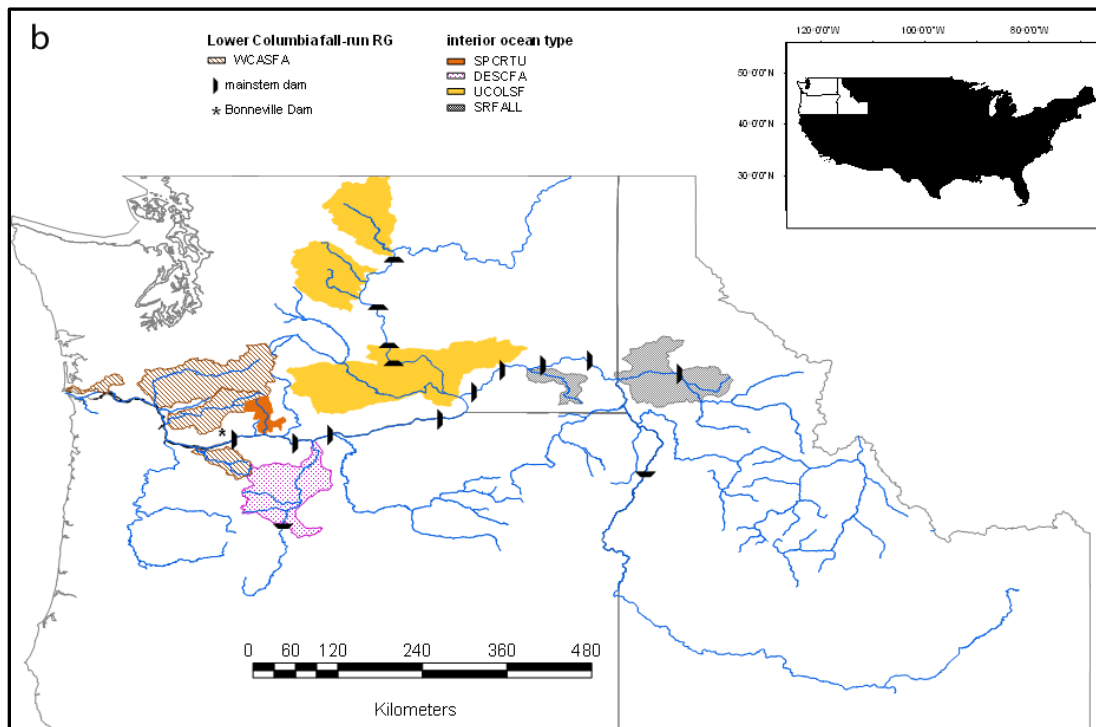
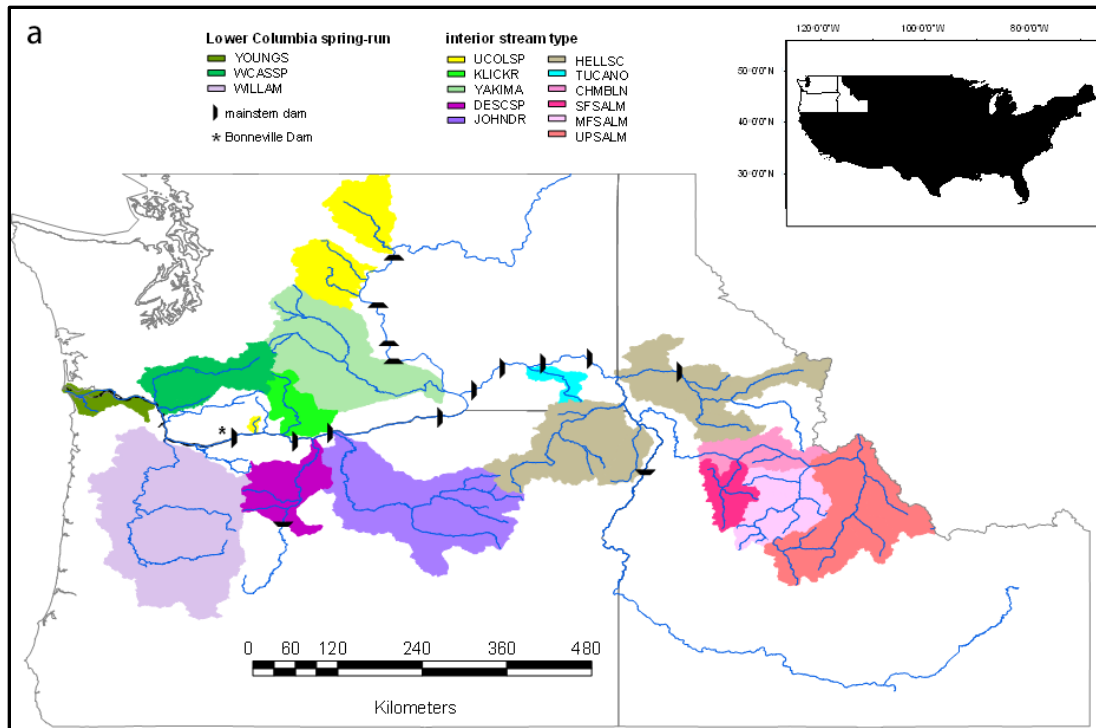


Figure 2: Map of Chinook salmon GSI reporting groups for a) Lower Columbia (LC) and interior stream type (ST) lineage, and b) interior ocean type (OT) lineage.

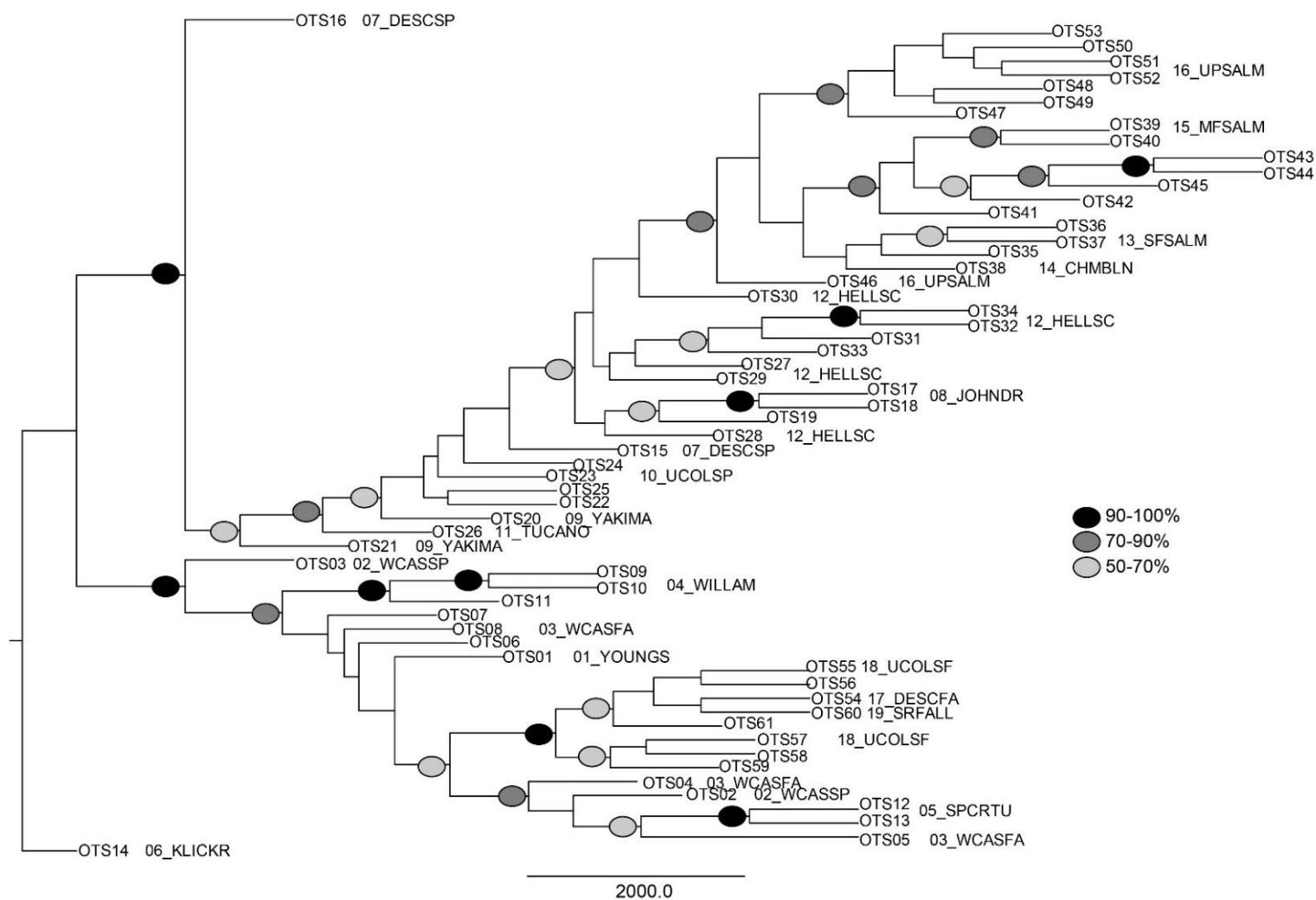


Figure 3: Neighbor-joining tree of Chinook salmon baseline populations using Nei's 1972 genetic distance of 179 SNP loci. The clusters are labeled with names of reporting groups used to aggregate the collections based on a combination of factors including genetic similarity, life history, and geographic proximity. Bootstrap support is shown with shaded ovals (Source: Hess et al. 2015).

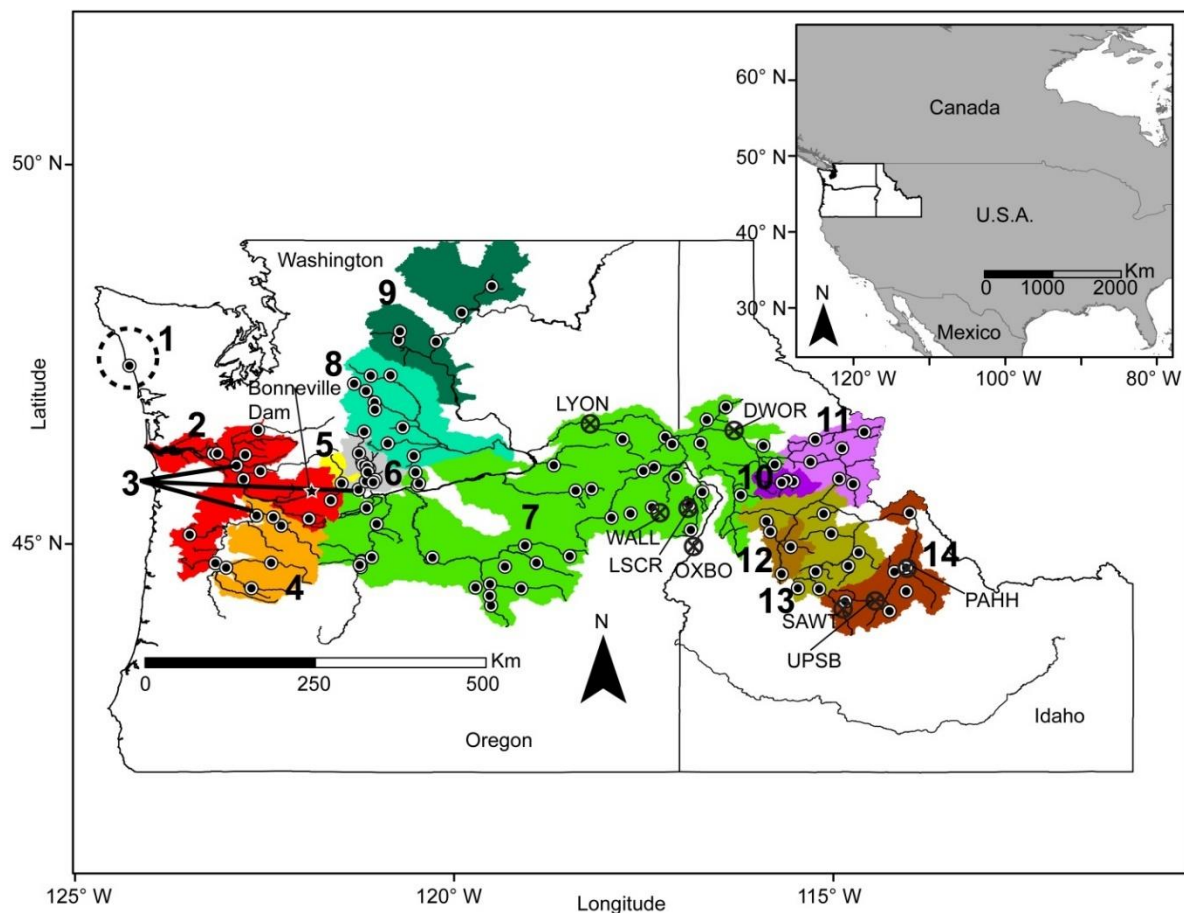


Figure 4: Geographic distribution of collections represented in the Columbia River steelhead GSI and PBT genetic baselines. The shape overlay represents the geographic extent of the following 14 reporting groups in the GSI baseline: 1) Quinault (WCOAST), 2) lower Columbia River (LOWCOL), 3) Skamania hatchery releases at three sites in lower Columbia River, Willamette River, and Klickitat River (SKAMAN), 4) Willamette River (WILLAM), 5) Big White Salmon River (BWSALM), 6) Klickitat River (KLICKR), 7) middle Columbia River, Grande Ronde River, Imnaha River, lower Snake River, lower Clearwater River, and lower Salmon River (MGILCS), 8) Yakima River (YAKIMA), 9) upper Columbia River (UPPCOL), 10) South Fork Clearwater River (SFCLWR), 11) upper Clearwater River (UPCLWR), 12) South Fork Salmon River (SFSALM), 13) Middle Fork Salmon River (MFSALM), and 14) upper Salmon River (UPSALM). There are 116 collections (filled circles) categorized into reporting groups. The PBT baseline is indicated as 8 stocks (crossed circles) corresponding to the following sites where fish are collected and spawned for broodstock: Lyons Ferry Hatchery (LYON), Wallowa (WALL), Little Sheep Creek (LSCR), Oxbow Hatchery (OXBO), Dworshak Hatchery (DWOR), upper Salmon River B-run (UPSB), Sawtooth Hatchery (SAWT), and Pahsimeroi Hatchery (PAHH). Bonneville Dam (star) is the site where fish were non-lethally sampled for the mixed-stock analysis.

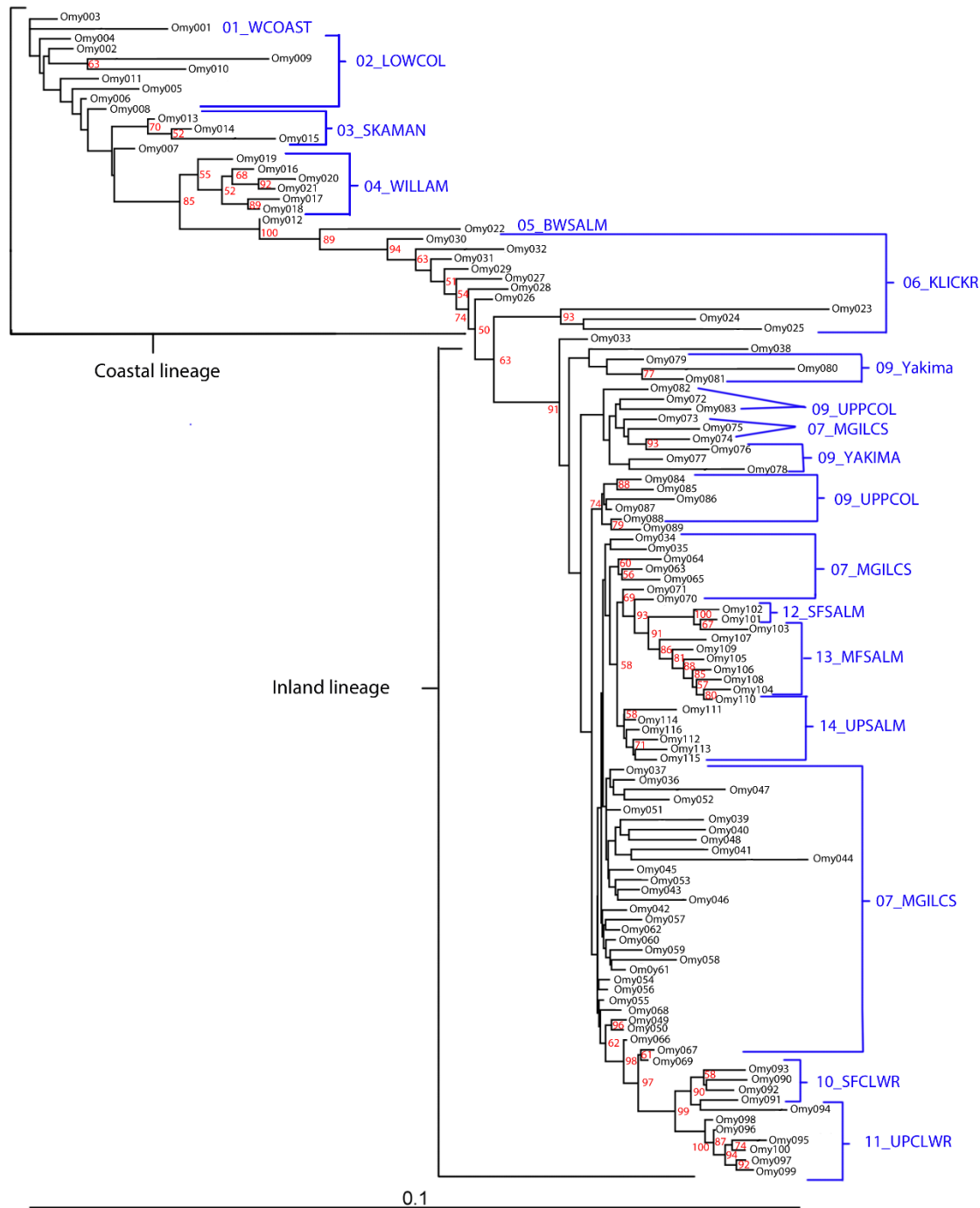


Figure 5: Neighbor-joining tree of steelhead baseline populations in GSI baseline v.3.3 (186 SNPs) using Nei's 1972 genetic distance. Bootstrap values (in red) $\geq 50\%$ (based on 1000 bootstraps) are shown. Reporting group names (in blue) are provided and are clustered by lineage (i.e, coastal or inland).

720 Table 3: Chinook salmon PBT baselines throughout the Columbia River basin. Chinook salmon hatchery programs are shown, along with run type, lineage and years of
721 availability (X). Cells marked with an asterisks (*) indicate collections that are not represented in our PBT baseline, and blank cells indicate collections that may be available but
722 have not yet been genotyped.

Hatchery	Code	Run type	Lineage	Year								
				2008	2009	2010	2011	2012	2013	2014	2015	2016
Clearwater Fish Hatchery	OtsCLWH	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Clearwater Fish Hatchery - Powell Facility	OtsPOWP	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Dworshak National Fish Hatchery	OtsDWOR	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Catherine Creek	OtsCTHW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Grande Ronde	OtsGRUW	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Imnaha River	OtsIMNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Lookingglass Creek	OtsLOOK	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Lookingglass Fish Hatchery - Lostine River	OtsLSTW	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	OtsLYON	Spring	Interior stream type	*	*	*	*	X	X	X	X	
Lyons Ferry Fish Hatchery - Tucannon River	OtsTUCW ^a	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	OtsLYON_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X	X
McCall Fish Hatchery - Johnson Creek	OtsJHNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X	X
McCall Fish Hatchery - South Fork Salmon	OtsMCCA	Spring	Interior stream type	X	X	X	X	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Fall)	OtsNPFH_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Spring)	OtsNPFH	Spring	Interior stream type	X	X	X	X	X	X	X	X	
Pahsimeroi Fish Hatchery	OtsPAHH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X
Rapid River Fish Hatchery	OtsRAPH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery	OtsSAWT	Spring	Interior stream type	X	X	X	X	X	X	X	X	X
Big Creek Hatchery	OtsBIG	Fall	Interior ocean type	*	*	*	*	*	*	*	X	X
Carson National Fish Hatchery	OtsCAR	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Chief Joseph Hatchery (Spring)	OtsCJH_sp	Spring	Interior stream type	*	*	*	*	*	*	X	X	X
Chief Joseph Hatchery (Summer/Fall)	OtsCJH_sufa	Summer	Interior ocean type	*	*	*	*	*	X	X	X	X
Cowlitz Salmon	OtsCOW	Spring	Interior stream type	*	*	*	*	*	*	*	*	*
Eastbank Fish Hatchery	OtsEASTBK	Summer	Interior ocean type	*	*	*	*	X	*	*	*	*
Entiat National Fish Hatchery	OtsENFH	Summer	Interior ocean type	*	*	*	*	*	X	X	X	X
Kalama Falls	OtsKAL	Spring	Interior stream type	*	*	*	*	*	*	*	*	*
Klickitat State Fish Hatchery	OtsKH	Spring	Interior stream type	X	X	X	X	X	X	X	X	X
Leavenworth National Fish Hatchery	OtsLNFH	Spring	Interior stream type	*	*	*	*	*	X	X	X	X
Lewis River	OtsLEW	Spring	Interior stream type	*	*	*	*	*	*	*	*	*
Little White Salmon National Fish Hatchery (Fall)	OtsLWS_sufa	Fall	Interior ocean type	*	*	*	*	*	X	X	X	X
Little White Salmon National Fish Hatchery (Spring)	OtsLWS_sp	Spring	Interior stream type	*	*	*	*	*	X	X	X	X
Methow State Fish Hatchery	OtsMETH	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Parkdale Fish Facility	OtsPFF	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Priest Rapids Hatchery	OtsPRH	Fall	Interior ocean type	*	*	*	*	X	X	X	X	X
Round Butte Fish Hatchery	OtsRB	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Ringold Springs State Hatchery	OtsRGS	Fall	Interior ocean type	*	*	*	*	*	*	*	*	X
Spring Creek NFH	OtsSPCR	Fall	Interior ocean type	*	*	*	*	*	*	*	X	X
Toutle	OtsTOU	Fall	Interior ocean type	*	*	*	*	*	*	*	*	*
Umatilla Fish Hatchery (Fall)	OtsUMA_sufa ^b	Fall	Interior ocean type	*	*	*	*	X	X	X	X	X
Umatilla Fish Hatchery (Spring)	OtsUMA_sp	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Washougal	OtsWAS	Fall	Interior ocean type	*	*	*	*	*	*	*	*	*
Warm Springs National Fish Hatchery	OtsWSNFH	Spring	Interior stream type	*	*	*	*	X	X	X	X	X
Wells Fish Hatchery	OtsWELLS	Summer	Interior ocean type	*	*	*	*	X	X	X	X	X
Winthrop National Fish Hatchery	OtsWTP	Spring	Interior stream type	*	*	*	*	*	X	X	X	X
Yakima Nation Prosser Hatchery	OtsPRO	Fall	Interior ocean type	*	*	*	*	X	X	*	X	X
Yakima River Roza Dam-Integrated	OtsYRint	Spring	Interior stream type	*	*	*	*	X	*	X	X	X
Yakima River Roza Dam-Segregated	OtsYRseg	Spring	Interior stream type	*	*	*	*	X	*	*	X	X

723 X – Tissues genotyped using 298 SNPs

724 X – Tissues genotyped using 96 SNPs

725 X – Data not yet available

726 ^aLyons Ferry stock consolidated under 'OtsLYON' starting in 2012

^bUmatilla fall stock spawned at Little White Salmon Hatchery starting in 2015; not distinguished from LWS stock

*Broodstock not sampled

727

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Table 4: Results of the accuracy testing for the Chinook salmon PBT baseline. Collections and their respective lineage/run type are shown along with the PBT baselines available for their assignment. The total number of successful assignments, and the number that assigned to their expected brood stock are displayed, as well as the number of unexpected assignments and their sources.

Collections	Lineage/Run type	PBT Baselines available					Samples	Total Assignments	Percent Assigned	Assigned to Expected Broodstock		Assigned to Unexpected Broodstock	Source(s)
		2011	2012	2013	2014	2015				N	Proportion	N	
Lyons Ferry 2016	Interior ocean type/Fall	X	X	X	X	X	1933	1165	60.27	1154	0.99	11	OtsLWS13_sufa(2); OtsLWS14_sufa(1); OtsPRH12(1); OtsPRH14(1); OtsUMA12_sufa(5); OtsUMA13_sufa(1)
Nez Perce 2016	Interior ocean type/Fall	X	X	X	X	X	752	471	62.63	461	0.98	10	OtsPRH14(1); OtsUMA12_sufa(9)
Sawtooth 2016	Interior stream type/Spring	X	X	X	X	X	1058	1003	94.80	1002	1.00	1	OtsPAHH11S(1)
McCall 2015 (Johnson Creek)	Interior stream type/Spring, Summer	X	X	X	X	X	70	3	4.29	3	1.00	0	
McCall 2016 (South Fork Salmon)	Interior stream type/Spring	X	X	X	X	X	843	790	93.71	789	1.00	1	OLtsLOOK12S(1)
Klickitat 2016	Interior stream type/Spring	X	X	X	X	X	476	282	59.24	280	0.99	2	OtsCLWH12S(1); OtsPOWP12S(1)
Round Butte 2016	Interior stream type/Spring		X	X	X	X	313	250	79.87	234	0.94	16	OtsDWOR11S(1); OtsDWOR12S(4); OLtsLOOK12S(3); OtsMCCA11S(2); OtsMCCA12S(2); OtsPAHH11S(2); OtsSAWT12S(2)

731

Chinook (94 SNPs vs 298 SNPs)

We compared the PBT assignment accuracy of the 94 SNP and the 298 SNP panels using the 2016 broodstock collections for the Lyons Ferry and Nez Perce programs. These were chosen because they have been genotyped for the 298 SNPs since 2011, and represent the only collections where such a comparison was possible (Table 3). After converting these collections to unknown samples, we conducted PBT assignment with the 94 SNP and 298 SNP panels, and using brood years 2011-2015 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341). Results using the 94 SNP panel are presented in the preceding section, and we don't recapitulate them here except to note that 1636 individuals were assigned via PBT and the average LOD-score and FDR for those individuals was 18.60 and 0.10, respectively. For the 298 SNP panel, we observed PBT assignments for 1657 individuals, and the average LOD-score and FDR were 28.34 and 0.03 (Table 5). Thus, the 298 SNP panel did appear to assign additional fall Chinook salmon via PBT, and provide greater confidence in PBT assignment over the 94 SNP panel, as reflected in higher LOD-scores and lower FDR values. This test will need to be repeated on collections from the interior stream type lineage as the 298 SNP panel is genotyped for additional programs in the future.

Steelhead (95 SNPs)

To test the accuracy of the steelhead PBT baseline (95 SNPs) in assigning known samples to their hatchery brood of origin, we selected five hatchery collections from 2016 and converted them to unknown samples. These hatchery collections were represented by two inland lineages from the Snake River (i.e., Dworshak Hatchery and Wallowa Hatchery), two inland lineages from the Columbia River (i.e., Umatilla Hatchery and Winthrop Hatchery), and one coastal lineage from the Columbia River (i.e., Parkdale Fish Facility). These collections were chosen because of the availability of PBT baselines in previous years that these samples could be assigned to (Table 6). For the interior lineage from the Snake River (i.e., Dworshak Hatchery and Wallowa Hatchery), PBT baselines extend as far back as 2008 and 2009, respectively, that allow us to assign fish from age classes 3, 4 and 5. However, for the interior and coastal lineage from the Columbia River (i.e., Umatilla Hatchery, Winthrop Hatchery, and Parkdale Fish Facility) our baselines extend to 2012 and only permit the assignment of age classes 3 and 4. After converting 2016 hatchery returns at each of these facilities to unknown samples, we conducted PBT assignments using brood years 2011-2015 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341). The expectation was that hatchery origin fish returning to each facility should assign to parent broodstock in the previous generation from the same hatchery. As described above, not all age classes were represented in PBT baselines for each program, so we expected missing assignments for certain

stocks. Even when all age classes are available, tagging rates may be lower than 100% that would also result in unassigned offspring. Further, some hatchery programs integrate natural origin fish, so these fish would also not be expected to assign to parent broodstock in the previous generation. Given that some offspring did not have true parents in the PBT baseline, this approach allowed us to test for false positive assignments in addition to broodstock assignment accuracy. Only those individuals that had a LOD-score ≥ 14 and a False Discovery Rate (FDR) ≤ 0.01 were considered to be successfully ‘assigned’, while all others were considered ‘unassigned’.

Dworshak Hatchery & Wallowa Hatchery:

The Dworshak and Wallowa samples from 2016 had the greatest percentage of successful assignments (i.e., 89% and 88%, respectively). This reflects the availability of parental baselines for assignment of all the age classes that are anticipated to be encountered in these samples. Of the offspring that were successfully assigned to parents, 100% of the Wallowa samples and 100% of the Dworshak samples from 2016 assigned to their expected broodstock (Table 7).

Umatilla Hatchery, Winthrop Hatchery & Parkdale Facility:

Since the PBT baseline for these facilities only extended to BY2012 and offspring from 2016 were tested, we only expected to assign 3- and 4-year old fish to parents. Of the offspring tested, 29% of the Umatilla and 5% of the Winthrop samples from 2016 were successfully assigned, while none of the Parkdale samples from 2016 were successfully assigned. For the Umatilla and Winthrop programs, this marks a 10% and 5% increase, respectively, in successful PBT assignments over a similar analysis conducted using 2015 broodstock from these programs, and reflects the expansion of our PBT baselines. We anticipate that continued expansion of our PBT baseline will further improve assignments as more age classes are represented. For Parkdale, this program typically has largely natural origin fish, so we would not expect assignments to broodstock parents. (The exact proportion of hatchery origin fish for the Umatilla and Parkdale collections from 2016 was unknown since it was not recorded at time of sampling.) Of the 3- and 4-year old Umatilla fish that were successfully assigned, 95% were assigned to their expected broodstock (Table 7). The single Umatilla sample that was assigned to an unexpected broodstock came from the Wallowa Hatchery in 2012, and likely represents a stray individual. The four Winthrop samples assigned to an unexpected broodstock came from the Okanogan stock at the Wells Hatchery in 2013, and reflects the transfer of Wells Hatchery fish to the Winthrop program. For all stocks, false-positive assignments (i.e., assignments to incorrect parents) were highly unlikely but it is possible that the few stray fish observed were actually mis-assigned.

Steelhead (95 SNPs vs 186 SNPs)

We compared the PBT assignment accuracy of the 95 SNP and the 186 SNP panels using the 2016 broodstock collections for the Umatilla, Winthrop and Parkdale programs. These were

chosen because they have been genotyped for the 186 SNPs since 2012, and represent the only collections where such a comparison was possible (Table 6). After converting these collections to unknown samples, we conducted PBT assignment with the 95 SNP and 186 SNP panels, and using brood years 2011-2015 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341). Results using the 95 SNP panel are presented in the preceding section, and we don't recapitulate them here except to note that 27 individuals were assigned via PBT and the average LOD-score and FDR for those individuals was 21.48 and 0.00, respectively (Table 8). For the 186 SNP panel, we observed PBT assignments for 25 individuals (two individuals had FDR >0.01 and were not considered 'assigned'), and the average LOD-score and FDR were 25.97 and 0.00 (Table 8). Thus, the 186 SNP panel did appear to provide greater confidence in PBT assignment over the 95 SNP panel, as reflected in higher LOD-scores, but this test will need to be repeated as the 186 SNP panel is genotyped for additional programs in the future.

Table 5: Comparison of PBT assignment accuracy for Chinook salmon using 94 SNPs and 298 SNPs.

No. markers	N	Average LOD	Average FDR
94 SNPs	1636	18.60	0.10
298 SNPs	1657	28.34	0.03

825 Table 6: Steelhead PBT baselines throughout the Columbia River basin. Steelhead hatchery programs are shown, along with run type, lineage and years of
826 availability (X). Cells marked with an asterisks (*) indicate collections that are not represented in our PBT baseline.

Hatchery	Code	Run type	Lineage	Year									
				2008	2009	2010	2011	2012	2013	2014	2015	2016	
Dworshak National Fish Hatchery	OmyDWOR	Unknown	Interior	X	X	X	X	X	X	X	X	X	
Little Sheep Creek Hatchery	OmyLSCR	Summer	Interior	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery- Touchet	OmyTOUW ^a	Summer	Interior	*	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery	OmyLYON ^b	Unknown	Interior	*	X	X	X	X	N/A	N/A	N/A	N/A	
Lyons Ferry Fish Hatchery - Grande Ronde	OmyCGRW ^a	Summer	Interior	X	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery - Tucannon	OmyTUCW ^a	Summer	Interior	*	X	X	X	X	X	X	X	X	
Lyons Ferry Fish Hatchery - Wallowa	OmyWALW	Summer	Interior	*	*	*	*	*	*	*	X	X	
Oxbow	OmyOXBO	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery	OmySAWT	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery - East Fork Salmon	OmyEFSW ^c	Summer	Interior	X	X	X	X	X	X	X	X	X	
Sawtooth Fish Hatchery - Squaw Creek	OmySQUW ^d	Summer	Interior	X	X	X	X	X	X	X	X	X	
Pahsimeroi Fish Hatchery	OmyPAHH	Unknown	Interior	X	X	X	X	X	X	X	X	X	
Wallowa Fish Hatchery	OmyWALL	Summer	Interior	*	X	X	X	X	X	X	X	X	
Eastbank Hatchery	OmyEASTBK	Summer	Interior	*	*	*	*	X	X	X	X	*	
Methow Hatchery (Twisp)	OmyTWP	Summer	Interior	*	*	*	*	*	X	X	X	X	
Parkdale Fish Facility	OmyPFF	Winter	Coastal	*	*	*	*	X	X	X	X	X	
Round Butte Fish Hatchery	OmyRB	Summer	Interior	*	*	*	*	*	X	X	X	X	
Skamania Hatchery (Summer)	OmySKH_su ^e	Summer	Coastal	*	*	*	*	*	X	X	X	X	
Skamania Hatchery (Winter)	OmySKH_wi ^e	Winter	Coastal	*	*	*	*	*	X	X	X	X	
Umatilla Fish Hatchery	OmyUMA	Summer	Interior	*	*	*	*	X	X	X	X	X	
Wells Hatchery - Okanogan stock	OmyWEL_OKA	Summer	Interior	*	*	*	*	*	X	X	X	X	
Wells Hatchery - Omak stock	OmyWEL_OMA	Summer	Interior	*	*	*	*	*	*	*	X	X	
Winthrop National Fish Hatchery	OmyWTP	Summer	Interior	*	*	*	*	X	X	X	X	X	

827 X - Tissues genotyped using 96 SNPs

828 X - Tissues genotyped using 192 SNPs

829 X – Tissues genotyped using 269 SNPs

830 X – Tissues genotyped using 379 SNPs

831

832 N/A – Stock discontinued/non-existent

^aLyons Ferry stock consolidated under 'OmyLYON' starting in 2012

^bLyons Ferry stock discontinued starting in 2013

^cSawtooth stock consolidated under 'OmySAWT' from 2012-2013

^dSawtooth stock consolidated under 'OmySAWT' in 2012; renamed 'Upper Salmon B-run' (YFLW) and consolidated under 'OmyPAHH' starting in 2013

^eSkamania stock is collected late in calendar year, and is designated for the following broodyear (i.e., late 2012 collections are part of BY2013)

833

834 Table 7: Results of the accuracy testing for the steelhead PBT baseline. Collections and their respective lineage/run type are shown along with the PBT baselines available for their assignment.
835 The total number of successful assignments, and the number that assigned to their expected brood stock are displayed, as well as the number of unexpected assignments and their sources.

Collections	Lineage/Run type	PBT Baselines available					Samples	Total Assignments	Percent Assigned	Assigned to Expected Broodstock		Assigned to Unexpected Broodstock	Source(s)
		2011	2012	2013	2014	2015				N	Proportion	N	
Dworshak 2016	Interior/Unknown	X	X	X	X	X	1448	1285	89%	1285	1.0000	0	
Wallowa 2016	Interior/Unknown	X	X	X	X	X	449	394	88%	394	1.0000	0	
Umatilla 2016	Interior/Summer		X	X	X	X	78	23	29%	22	0.9565	1	OmyWALL12S (1)
Winthrop 2016	Interior/Summer		X	X	X	X	85	4	5%	0	0.0000	4	OmyWEL_OKA13 (4)
Parkdale 2016	Coastal/Winter		X	X	X	X	40	0	0%	0	0.0000	0	

836

837 Table 8: Comparison of PBT assignment accuracy for steelhead using 95 SNPs and 186 SNPs.

No. markers	N	Average LOD	Average FDR
95 SNPs	27	21.48	0.00
186 SNPs	25	25.97	0.00

838

Accuracy testing of the GSI baseline

To test the accuracy of the GSI baselines for Chinook salmon and steelhead, we conducted the self-assignment procedure implemented in Geneclass v.2.0 (Piry et al. 2004) using the ‘leave-one-out’ approach and the Bayesian resampling procedure of Rannala and Mountain (1997) to evaluate how well individuals could be assigned to their population and reporting group of origin. We examined the proportion of self-assignments for all individuals, but also examined how implementing a self-assignment probability threshold of ≥ 0.80 influenced overall self-assignments to reporting group of origin.

Chinook (179 SNPs)

For Chinook salmon, we used GSI baseline v3.1 that comprises 61 collections from throughout the Columbia River basin that are partitioned into 19 reporting groups (N=7083) (Figure 2). When considering the assignment of all individuals in the GSI baseline, 5820 (82%) of the 7083 individuals correctly assigned to their reporting group of origin (Table 9). Across all reporting groups, the proportion of correct assignments ranged from 0.52 (08_JOHNDR) to 1.0 (01_YOUNGS) (Figure 6a). Employing a self-assignment probability threshold of ≥ 0.80 reduced the number of fish assigned to 3000 individuals, but increased the proportion of correct assignments to 0.94 (n=2808). When implementing this threshold, the proportion of correct assignments across all reporting groups ranged from 0.79 (12_HELLSC) to 1.0 (01_YOUNGS, 04_WILLAM, 07_DECSP)(Figure 6b).

The highest proportion of correct assignments when considering all individuals was observed for Lower Columbia (LC) lineage reporting groups, and ranged from 0.83 (02_WCASSP) to 1.0 (01_YOUNGS) (Table 9; Figure 6a). Incorrect assignments for LC lineage populations were typically to other reporting groups within this lineage with the most common incorrect assignments to reporting groups that included collections from the same river (i.e., Cowlitz spring and fall run). However, six individuals were incorrectly assigned to the Interior Ocean Type (OT) lineage (18_UCOLSF; 19_SRFALL), and 12 individuals were assigned incorrectly to the Interior Stream Type (ST) lineage (06_KLICKR; 09_YAKIMA) (Table 9). Incorrect assignments to the 06_KLICKR reporting group are likely due introgression that has occurred between lineages in the Klickitat sub-basin (Hess et al. 2014).

The proportion of correct assignments for OT lineage reporting groups ranged from 0.74 (19_SRFALL) to 0.90 (17_DESCFA) (Table 9; Figure 6a). Incorrect assignments for OT lineage populations when considering all individuals were typically to other reporting groups within this lineage, or to LC lineage reporting groups (i.e., OTS54 – Upper Deschutes River fall run to 01_YOUNGS, 02_WCASSP, 03_WCASFA, and 04_WILLAM, and OTS55 Lower Yakima River fall run to 02_WCASSP). A single individual from OTS57 – Wenatchee River summer run incorrectly assigned to 06_KLICKR, and one individuals from OTS59 – Methow River summer run incorrectly assigned to 02_WCASSP (Table 9). These types of incorrect assignments were not as common as those to reporting group of the same run type.

The proportion of correct assignments for ST lineage reporting groups ranged from 0.52 (08_JOHNDR) to 0.94 (09_YAKIMA) (Table 9; Figure 6a). Incorrect assignments were

882 typically to other reporting groups within this lineage. However, we did observe instances where
883 ST lineage samples were assigned incorrectly to a LC lineage reporting group (i.e., OTS17 –
884 John Day River spring run to 02_WCASSP; OTS26 – Tucannon River spring run to
885 02_WCASSP; OTS27 – Wenaha River spring run to 01_YOUNGS and 02_WCASSP; OTS28 –
886 Lostine River spring run to 01_YOUNGS) (Table 9).

Table 9: Results of the leave-one-out analysis for the Chinook salmon GSI v3.1 baseline. Lineage, run type, reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of correct assignments to reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The proportion of correct assignments to reporting group for each population and reporting group (yellow shading) in the baseline is provided.

Lineage	Run type	RepGrp	PopID	Pop Name	N	Number assigned to reporting group																		Proportion assigned correct	
						01_YOUNGS	02_WCASSP	03_WCASFA	04_WILLAM	05_SPCRTU	06_KLICKR	07_DESCSP	08_JOHNDR	09_YAKIMA	10_UCOLSP	11_TUCANO	12_HELLSC	13_SFSALM	14_CHMBLN	15_MFSALM	16_UPSALM	17_DESCFA	18_UCOLSF		19_SRFALL
Lower Columbia	Spring	01_YOUNGS	OTS01	Youngs Bay fall-run	91	91																		1.0000	
			Reporting Group total		91	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0000
Lower Columbia	Spring	02_WCASSP	OTS02	Cowlitz R spring-run	90		80	9		1														0.8889	
			OTS03	Kalama R spring-run	83		63	2	6		11			1										0.7590	
			Reporting Group total		173	0	143	11	6	1	11	0	0	1	0	0	0	0	0	0	0	0	0	0	0.8266
Lower Columbia	Fall	03_WCASFA	OTS04	Cowlitz R fall-run	82		8	73	1															0.8902	
			OTS05	Elochoman R fall-run	86	8	1	61		15												1		0.7093	
			OTS06	Lewis R fall-run	93		2	91																0.9785	
			OTS07	NF Lewis fall-run	178			178																1.0000	
			OTS08	Sandy R fall-run	83			79	1													3		0.9518	
			Reporting Group total		522	8	11	482	2	15	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0.9234
Lower Columbia	Spring	04_WILLAM	OTS09	McKenzie R spring-run	78				78															1.0000	
			OTS10	N Santiam R spring-run	79				79															1.0000	
			OTS11	Sandy R spring-run	48		1	4	43															0.8958	
			Reporting Group total		205	0	1	4	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.9756
Lower Columbia	Fall	05_SPCRTU	OTS12	White Salmon fall-run	77			2		75														0.9740	
			OTS13	Spring Creek NFH tule fall-run	49					47													2	0.9592	
			Reporting Group total		126	0	0	2	0	122	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.9683
Interior stream type	Spring	06_KLICKR	OTS14	Klickitat R spring-run	84						76			5	1		2							0.9048	
			Reporting Group total		84	0	0	0	0	0	76	0	0	5	1	0	2	0	0	0	0	0	0	0	0.9048
Interior stream type	Spring	07_DESCSP	OTS15	Shitike R spring-run	93						1	80	1		6		1	2		1	1			0.8602	
			OTS16	Warm Springs R spring-run	90								88			1		1						0.9778	
			Reporting Group total		183	0	0	0	0	0	1	168	1	0	7	0	2	2	0	1	1	0	0	0	0
Interior stream type	Spring	08_JOHNDR	OTS17	John Day R spring-run	78		1						42	3	5		20	2		1	4			0.5385	
			OTS18	Middle Fork John Day R spring-run	47							1	32			1		12	1					0.6809	
			OTS19	North Fork John Day R spring-run	42								12	1	8	1	12	5		3				0.2857	
			Reporting Group total		167	0	1	0	0	0	0	1	86	4	14	1	44	8	0	4	4	0	0	0	0
Interior stream type	Spring	09_YAKIMA	OTS20	American R spring-run	76									76										1.0000	
			OTS21	Cle-Elum spring-run	88								1	78	3	2	3			1				0.8864	
			Reporting Group total		164	0	0	0	0	0	0	0	1	154	3	2	3	0	0	1	0	0	0	0	0
Interior stream type	Spring	10_UCOLSP	OTS22	Winthrop NFH spring-run	82								6		55		11	4		2	4			0.6707	
			OTS23	little White Salmon R spring-run	93							1	2		74		9	3			4			0.7957	
			OTS24	Wenatchee R spring-run	109										85				2	5				0.7798	
			OTS25	Entiat R spring-run	98						1		5	2	64	1	16	1		2	6			0.6531	
			Reporting Group total		382	0	0	0	0	0	1	1	13	2	278	1	50	11	0	6	19	0	0	0	0
Interior stream type	Spring	11_TUCANO	OTS26	Tucannon R spring-run	81		1							2	2	65	9	1		1				0.8025	
			Reporting Group total		81	0	1	0	0	0	0	0	0	2	2	65	9	1	0	1	0	0	0	0	0.8025
Interior stream type	Spring	12_HELLSC	OTS27	Wenaha R spring-run	179	3	1				5	1	17	2	14	4	121	5		1	5			0.6760	
			OTS28	Lostine R spring-run	213	1								19	2	5	1	171	3		4	7		0.8028	
			OTS29	Grande Ronde R spring-run	313						4	2	33	2	26	2	207	11		10	16			0.6613	
			OTS30	Imnaha R spring-run	96							1	1		3		69	5		6	11			0.7188	
			OTS31	Lolo Cr spring-run	89								4		14	1	58	3	1	1	7			0.6517	
			OTS32	Red R spring-run	221						1	1	9		22		161	4		6	17			0.7285	
			OTS33	Powell R spring-run	56								1		12		35	1		4	3			0.6250	
			OTS34	Red R weir spring-run	91										1		84	2		1	3			0.9231	
			Reporting Group total		1258	4	1	0	0	0	10	5	84	6	97	8	906	34	1	33	69	0	0	0	0
Interior stream type	Spring	13_SFSALM	OTS35	South Forth Salmon R spring-run	139							4		4		16	74	3	16	22			0.5324		
			OTS36	Johnson Cr spring-run	137							1				6	106	1	12	11			0.7737		
			OTS37	Secesh R spring-run	252							1		6		13	208	1	16	7			0.8254		
			Reporting Group total		528	0	0	0	0	0	0	1	5	0	10	0	35	388	5	44	40	0	0	0	0.7348
Interior stream type	Spring	14_CHMBLN	OTS38	Chamberlain Cr spring-run	219		0	0	0	0	0	0	1	0	1	0	12	5	188	5	6			0.8584	
			Reporting Group total		219	0	0	0	0	0	0	0	1	0	1	0	12	5	188	5	6	0	0	0	0.8584
Interior stream type	Spring	15_MFSALM	OTS39	Big Cr spring-run	139								3				13	5	2	105	11			0.7554	
			OTS40	Camas Cr spring-run	55												2	1	3	48	1			0.8727	
			OTS41	Loon Cr spring-run	107							1	1		1		4	4	2	93	1			0.8692	
			OTS42	Sulphur Cr spring-run	94								1		1		3	2		80	7			0.8511	
			OTS43	Bear Valley Cr spring-run	135											1	1		128	5			0.9481		
			OTS44	Capehorn Cr spring-run	214								1		2		6	10	2	189	4			0.8832	
			OTS45	Marsh Cr spring-run	228									2		3		3	11		196	13			0.8596
			Reporting Group total		972	0	0	0	0	0	0	0	1	8	0	7	0	32	34	9	839	42	0	0	0
Interior stream type	Spring/Summer	16_UPSALM	OTS46	North Fork Salmon R spring-run	55										1		7	1		2	44			0.8000	
			OTS47	Lemhi R spring-run	96								1				7	4		8	76			0.7917	
			OTS48	Pahsimeroi R spring-run	92											1	6		3	81				0.8804	
			OTS49	East Fork Salmon R spring-run	286										3		11	5		13	254			0.8881	
			OTS50	Salmon R spring-run	83										2			2		7	72			0.8675	

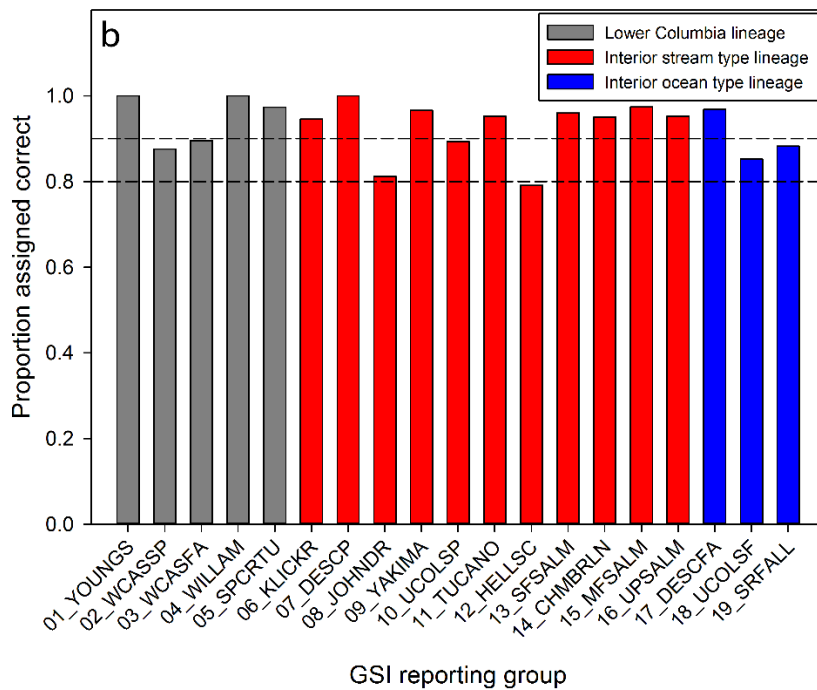
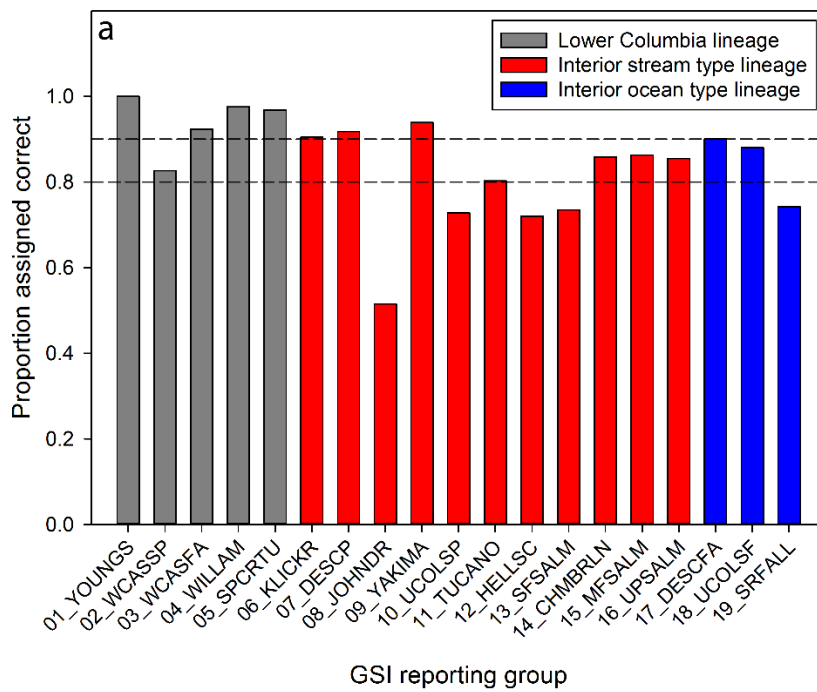


Figure 6: Proportion of Chinook salmon in leave-one-out tests that assigned correctly for each reporting group by lineage using a) all data, and b) $\geq 80\%$ self-assignment probability threshold. The dashed lines indicate 80% and 90% thresholds for correct assignment.

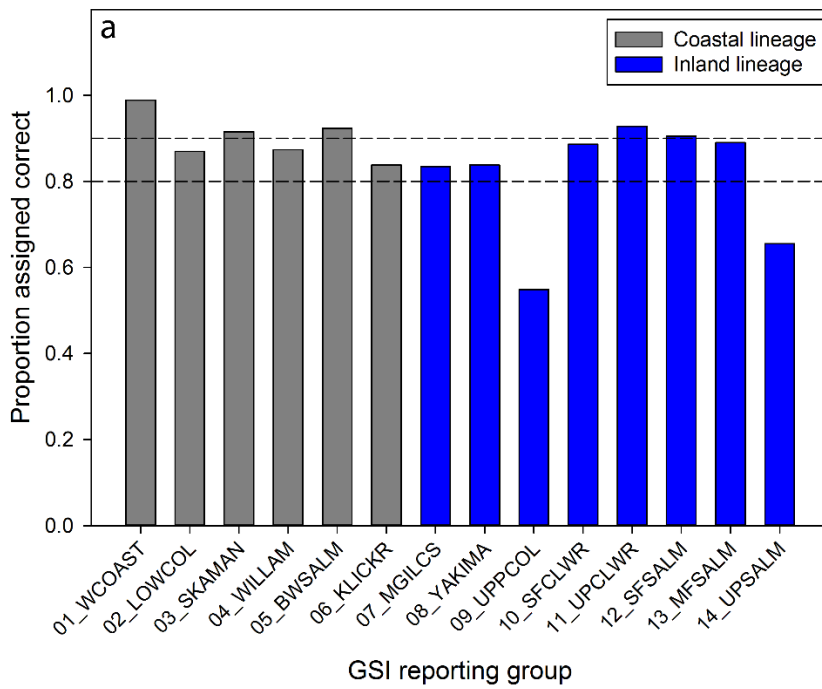
897 For steelhead, we used GSI baseline v3.3 that comprises 116 collections from throughout
898 the Columbia River basin that are partitioned into 14 reporting groups (N= 9991) (Figure 4).
899 When considering the assignment of all individuals in the GSI baseline, 8364 (84%) of the 9991
900 individuals correctly assigned to their reporting group of origin (Table 10). Across all reporting
901 groups, the proportion of correct assignments ranged from 0.55 (09_UPPCOL) to 0.99
902 (01_WCOAST) (Figure 7a). Employing a self-assignment probability threshold of ≥ 0.80 reduced
903 the number of fish assigned to 3989 individuals, but increased the proportion of correct
904 assignments to 0.94 (n=3758). When implementing this threshold, the proportion of correct
905 assignments across all reporting groups ranged 0.77 (09_UPPCOL) to 1.00 (01_WCOAST)
906 (Figure 7b). When considering all individuals, the proportion of correct assignments for most
907 reporting groups was >0.80 ; the two exceptions being 09_UPPCOL (0.55) and 14_UPSALM
908 (0.66) (Table 10; Figure 7a) with the majority of incorrect assignments to the 07_MGILCS
909 reporting group. The proportion of correct assignments for inland lineage reporting groups
910 ranged from 0.55 (09_UPPCOL) to 0.93 (11_UPCLWR) (Table 10). These results support the
911 inclusion of a hierarchical approach to improve stock ID results by resolving assignment to the
912 MGILCS reporting group with more markers as discussed in Section 2.

913 We detected samples (n=28, 1.5%) from the coastal lineage (i.e., 01_WCOAST –
914 06_KLICKR reporting groups) that were incorrectly assigned to reporting groups of the inland
915 lineage (i.e., 07_MGILCS – 14_UPSALM reporting groups (Table 10). We similarly detected
916 samples (n=29, 0.34%) from reporting groups from the inland lineage were incorrectly assigned
917 to coastal lineage reporting groups (Table 10). Incorrect assignments were typically distributed
918 across multiple reporting groups, but the largest proportion of incorrect assignments were
919 consistently to three reporting groups (07_MGILCS, 06_KLICKR, and 05_BWSALM).
920 Samples from the geographically large 07_MGILCS reporting group were incorrectly assigned
921 to every reporting group except 01_WCOAST, 03_SKAMAN, and 04_WILLAM (Table 10).

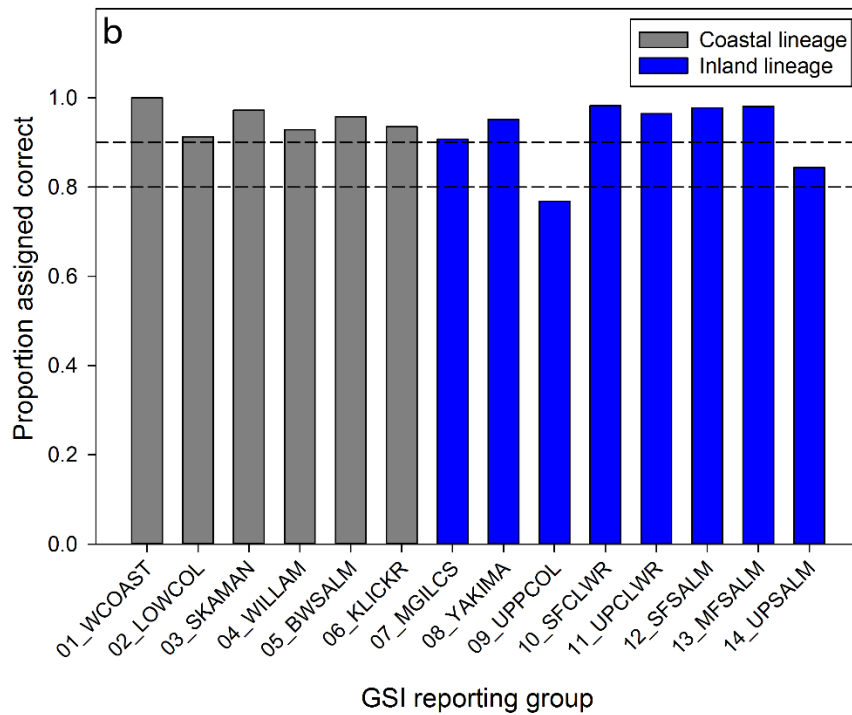
922 Table 10: Results of the leave-one-out analysis for the steelhead GSI v3.3 baseline. Reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of correct assignments to
923 reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The proportion of correct assignments to reporting group for each
924 population and reporting group (yellow shading) in the baseline is provided.

Lineage	Reporting Group	PopID	Pop Name	N	Number assigned to reporting group														Proportion assigned correct	
					01_WCOAST	02_LOWCOL	03_SKAMAN	04_WILLAM	05_BWSALM	06_KLICKR	07_MGILCS	08_YAKIMA	09_UPPCOL	10_SFCLWR	11_UPCLWR	12_SFSALM	13_MFSALM	14_UPSALM		
Coastal	01_WCOAST	OMY001	Quinault River	89	88	1													0.989	
		Reporting Group total			89	88	1	0	0	0	0	0	0	0	0	0	0	0	0.989	
Coastal	02_LOWCOL	OMY002	Mill Creek	43	3	40													0.930	
		OMY003	Germany Creek	47	1	43	1	2											0.915	
		OMY004	Coweeman River	45		42	3												0.933	
		OMY005	Cowliltz River	94		93	1												0.989	
		OMY006	Kalama River - winter run	94		80	12	1			1								0.851	
		OMY007	East Fork Lewis River	77		57	14	5					1						0.740	
		OMY008	North Fork Lewis River	94	1	88	2	2	1										0.936	
		OMY009	Luckiamute River	26		25		1											0.962	
		OMY010	Willamina Creek	30		27		3											0.900	
		OMY011	Still Creek	28		20	4	4											0.714	
		OMY012	East Fork Hood River	52		33	1	6		12									0.635	
		Reporting Group total			630	5	548	38	24	1	12	1	0	0	1	0	0	0	0	0.870
Coastal	03_SKAMAN	OMY013	Kalama River - summer run	94	1	29	64												0.681	
		OMY014	Clackamas River - summer run	59		3	55	1											0.932	
		OMY015	Klickitat-Skamania - summer run	249			249												1.000	
		Reporting Group total			402	1	32	368	1	0	0	0	0	0	0	0	0	0	0.915	
Coastal	04_WILLAM	OMY016	Clackamas River - winter run	92		3	3	84		1	1								0.913	
		OMY017	North Fork Eagle River	43		9		34											0.791	
		OMY018	Eagle River	47		7		40											0.851	
		OMY019	Little Rock/Mad River	50		1	7	41	1										0.820	
		OMY020	North Fork Santiam River	39		1		38											0.974	
		OMY021	South Fork Santiam River/Wiley	93		2	10	81											0.871	
		Reporting Group total			364	0	23	20	318	1	1	1	0	0	0	0	0	0	0.874	
Coastal	05_BWSALM	OMY022	Big White Salmon River	78		3			72	3									0.923	
		Reporting Group total			78	0	3	0	0	72	3	0	0	0	0	0	0	0	0.923	
Coastal	06_KLICKR	OMY023	Upper Trout Creek	31						30				1					0.968	
		OMY024	Suveyors Creek	25		1				24									0.960	
		OMY025	Snyder Creek	26						25				1					0.962	
		OMY026	Lower Summit Creek	37		1	1			29	3		1			1		1	0.784	
		OMY027	Lower Trout Creek	22		1	1			17	3								0.773	
		OMY028	Lower White Creek	29		1				22	4		1	1					0.759	
		OMY029	Little Klickitat River	30		2				25	2								0.833	
		OMY030	Dead Canyon Creek	20			5			14	1								0.700	
		OMY031	Bowman Creek	37		3	1			29	2								0.784	
		OMY032	Swale Creek	21		1		1		18								2	0.857	
		Reporting Group total			278	0	10	8	1	1	233	15	0	2	3	0	1	0	4	0.838
		Inland	07_MGILCS	OMY033	Fifteenmile Creek	91		1				4	75	4	4					3
OMY034	Pelton			45						1		27	3	2	1		2	2	7	0.600
OMY035	Shitike Creek			31							1	27		1					2	0.871
OMY036	Buck Hollow Creek			63								51	2	4					6	0.810
OMY037	Deschutes River-Trout Creek			57								47	1	4				1	4	0.825
OMY038	Deschutes River-upper mainstem			61								59				1		1		0.967
OMY039	Beech			21								19		1					1	0.905
OMY040	John Day River - upper mainstem			34								33		1						0.971
OMY041	Baldy			25								25								1.000
OMY042	John Day River - lower mainstem			44								36	1	1	1	1			4	0.818
OMY043	John Day River - upper middle fork			107								102	1	2				2		0.953
OMY044	Granite			18								18								1.000
OMY045	North Fork John Day River			56								50	2	2			1		1	0.893
OMY046	Big Wall			22								20		1			1			0.909
OMY047	Deer Creek			18								18								1.000
OMY048	Murderers Creek			18								18								1.000
OMY049	Rock Creek			126								105	2	4	2	3		1	9	0.833
OMY050	Squaw Creek			136							1	103	3	7	5	2		2	13	0.757
OMY051	Iskuulpa Creek			148							1	124	7	6			1		9	0.838
OMY052	Umatilla River			34								30	1	1		1		1		0.882
OMY053	Touchet River			86								84						1	1	0.977
OMY054	Alpowa Creek			98							1	67	4	18	4	1			3	0.684
OMY055	Asotin Creek			194								134	9	28	2	1	1	2	17	0.691
OMY056	Tucannon River			106								79	5	12	1		1	2	6	0.745
OMY057	Joseph Creek			97								89		4		1		1	2	0.918
OMY058	Little Minam River			48								43	3	1					1	0.896
OMY059	Menatchee Creek			73								67	2	1					3	0.918
OMY060	Upper Grand Ronde River			156								134	1	8			1	4	8	0.859
OMY061	Wallowa River			117			1			2		98	4	4			1		7	0.838
OMY062	Wenaha River			191							1	160		19			1	2	8	0.838
OMY063	Big Sheep Creek			184								166	1	5				5	7	0.902
OMY065	Lightning Creek			39								30	1	2		1		1	4	0.769
OMY066	Upper Imnaha River			53								43		4	1			3	2	0.811
OMY067	Big Bear Creek			250								217	1	11	5	6			10	0.868
OMY068	East Fork Potlatch River			158							1	136		4	10	4	2		1	0.861
OMY069	Lapwai Creek			158								134	2	11		2			9	0.848
OMY072	West Fork Potlatch River			84								72		3	7		1		1	0.857
OMY073	Little Salmon River			147								103	2	1	5		5	10	21	0.701
OMY074	Slate Creek			75								52		3			3	3	14	0.693
Reporting Group total				3469	0	2	0	0	3	10	2895	62	180	44	24	21	44	184	0.835	
Inland	08_YAKIMA	OMY075	Naches River - Rattlesnake Creek	36							7	27	2						0.750	
		OMY076	Naches River - Nile Creek	59							6	50	2				1		0.847	
		OMY077	Naches River - Pileup Creek	26							2	23						1	0.885	
		OMY078	Naches River - Quartz Creek	26							3	23							0.885	
		OMY079	North Fork Little Naches River	21							2	18	1						0.857	
		OMY080	Satus Creek	46							6	38				1		1	0.826	
		OMY081	Toppenish Creek	34							1	33							0.971	
		OMY081.2	Ahtanum Creek	40							1									

Lineage	Reporting Group	PopID	Pop Name	N	Number assigned to reporting group														Proportion assigned correct	
					01_WCOAST	02_LOWCOL	03_SKAMAN	04_WILLAM	05_BWSALM	06_KICKR	07_MGILCS	08_YAKIMA	09_UPPCOL	10_SFCLWR	11_UPCLWR	12_SFSALM	13_MFSALM	14_UPSALM		
Inland	09_UPPCOL	OMY082	Chiwaukum Creek	54						1	14	2	36					1	0.667	
		OMY083	Upper Chiwaukum Creek	29							8		15				3	3	0.517	
		OMY084	Nason River	21							6		15						0.714	
		OMY085	Entiat River	193					1		82	6	90	1	4	1	2	6	0.466	
		OMY086	Methow River	90							37	2	44		1			6	0.489	
		OMY088	Omak River	94					1		19	2	64			1		7	0.681	
		Reporting Group total			481	0	0	0	0	2	1	166	12	264	1	5	2	5	23	0.549
Inland	10_SFCLWR	OMY070	Lolo Creek	94							2		1	84	6			1	0.894	
		OMY090	Clear Creek	45						1	5			36	3				0.800	
		OMY091	Crooked River	136							4			125	7				0.919	
		OMY092	Newsome Creek	99										94	4			1	0.949	
		OMY093	Tenmile Creek	47							4			34	9				0.723	
		Reporting Group total			421	0	0	0	0	0	1	15	0	1	373	29	0	0	2	0.886
Inland	11_UPCLWR	OMY094	Bear Creek	70							1				68			1	0.971	
		OMY095	Lower Selway River	211							9		2	22	178				0.844	
		OMY096	Middle Lochsa River	147							6			4	136			1	0.925	
		OMY097	Middle Selway River	138							3		1	5	128	1			0.928	
		OMY098	Upper Lochsa River	129							1			6	122				0.946	
		OMY099	Upper Selway River	247							2		1	2	242				0.980	
		Reporting Group total			942	0	0	0	0	0	0	22	0	4	39	874	1	0	2	0.928
		Inland	12_SFSALM	OMY100	East Fork South Fork Salmon River	135							9		1			121	4	
OMY101	Secesh River			208							13		1			188	1	5	0.904	
OMY102	South Fork Salmon River			45							2	1				42			0.933	
Reporting Group total				388	0	0	0	0	0	0	24	1	2	0	0	351	5	5	0.905	
Inland	13_MFSALM	OMY103	Bear Valley Creek	173							6				1	166			0.960	
		OMY104	Big Creek	224							11			1		2	203	7	0.906	
		OMY105	Camas Creek	97							3	1				3	90		0.928	
		OMY106	Chamberlain Creek	189							16		1			1	166	5	0.878	
		OMY107	Loon Creek	131							6				1		123	1	0.939	
		OMY108	Marsh Creek	195							16	3	5		1	2	151	17	0.774	
		OMY109	Middle Fork Salmon River	227							15	1	1			2	201	7	0.885	
Reporting Group total			1236	0	0	0	0	0	0	73	5	7	1	2	11	1100	37	0.890		
Inland	14_UPSALM	OMY110	Herd Creek	85						1	19		4				2	59	0.694	
		OMY111	Lemhi River	86						1	1	13	4	3			3	61	0.709	
		OMY112	Morgan Creek	61						1		9		1		1		49	0.803	
		OMY113	North Fork Salmon River	100								44		4		1	3	47	0.470	
		OMY114	Pahsimeroi River	97					2		29	2	1				1	62	0.639	
		OMY115	Sawtooth Hatchery	319								100	1	4				2	212	0.665
		Reporting Group total			748	0	0	0	0	4	2	214	7	17	0	1	2	11	490	0.655



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927

928 Figure 7: Proportion of steelhead in leave-one-out tests that assigned correctly for each reporting
 929 group by lineage using a) all data, and b) $\geq 80\%$ self-assignment probability threshold. The
 930 dashed lines indicate 80% and 90% thresholds for correct assignment.

Parentage based tagging assignments of Chinook salmon in harvest mixtures

A summary of the Chinook harvest samples that were genotyped (derived from Table 1) is presented in Table 11. Of the 4,723 harvest Chinook analyzed, PBT identified 1,803 hatchery-origin individuals that could be confidently assigned back to 67 hatchery broodstock sources (i.e., 39 Snake River hatchery broodstocks and 28 Columbia River hatchery broodstocks) spawned in 2011-2014 (Table 12). The majority of PBT assigned individuals (77.76%) were from the 2012 brood year (i.e., 4-years-old), with a smaller fraction represented by 2-year old (brood year 2014; 0.11%), 3-year-old (brood year 2013; 7.32%) and 5-year-old (brood year 2011; 14.81%) fish. Of the 4-year-old fish, the majority (19.32%) were assigned to the Rapid River Fish Hatchery, followed by the Dworshak National Fish Hatchery (14.55%), and McCall Fish Hatchery (8.91%) – all from the Snake River drainage. The majority of 4-year-old fish assigned to Snake River hatchery broodstocks (68.76%), while 31.24% of PBT assigned 4-year-old fish assigned to Columbia River hatchery broodstocks (e.g., Carson National Fish Hatchery, Priest Rapids Hatchery, Warm Springs National Fish Hatchery).

Wind River spring-run Chinook salmon sport harvest

Both PBT and GSI assignments were used to analyze the Wind River spring-run Chinook salmon mark-selective sport fishery. We estimated stock composition to investigate how expansion of the Chinook salmon sport fishing “bubble” boundary around the mouth of the Wind River may be affecting proportions of non-local (i.e., Carson Hatchery) Chinook salmon that are harvested by using the context of stock proportions in other spring management period fisheries and at Bonneville Dam. We detected PBT assignments for 1/3 of the Chinook salmon harvested in the Wind River sport mark-selective fishery; Carson Hatchery 2012 (n=12), and Rapid River Hatchery 2013 (n=1). Our PBT baseline for Carson Hatchery broodstocks only extends back to 2012 (Table 3) and we were only likely to be able to assign 3-year old jacks and 4-year old adults from the 2016 fishery; the 2017 fishery is likely to be the first year in which 3- 4- and 5-year old fish may be assigned via PBT.

We also applied GSI to examine the stock composition of the Wind River fishery. We found that the 10_UCOLSP reporting group (includes upper Columbia River hatcheries as well as Carson Hatchery, Walla Walla Hatchery, and Umatilla Hatcheries) represented the greatest proportion of harvest (51.3%) followed to a much lesser extent by the 08_JOHNDR (2.56%) reporting group (Figure 8). Five (12.8%) of the Chinook salmon taken in the Wind River sport harvest could not be assigned using either PBT or GSI, and remained ‘unassigned’ (Figure 8).

967 Table 11: Summary of the Chinook salmon harvest samples by fishery, region, and origin in
 968 2016.

Fishery	Harvest Region	Origin	Management period		
			Spring	Summer	Fall
Lower River Commercial	Region A	Hatchery	472	0	0
		Wild	13	0	0
	Region B	Hatchery	439	0	0
		Wild	6	0	0
Lower River Test	Region B	Hatchery	324	0	0
		Wild	33	0	0
Lower River Sport	Region A	Hatchery	748	0	0
	Region B	Hatchery	985	0	0
Wind River Sport	Zone 6	Hatchery	39	0	0
Tribal Harvest	Zone 6	Hatchery	0	210	0
		Wild	0	221	0
Lower River Commercial	Region A	Hatchery	0	4	0
		Wild	0	178	0
	Region B	Hatchery	0	77	0
		Wild	0	127	0
Lower River Sport	Region A	Hatchery	0	108	0
	Region B	Hatchery	0	218	0
Tribal Harvest	Zone 6	Hatchery/Wild	0	0	373
Lower River Commercial	Region A	Hatchery	0	0	148

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Table 12: Summary information on the number and origin of PBT assigned Chinook salmon in 2016 fishery mixtures. HOR= hatchery origin (i.e., adipose-clipped), NOR=natural origin (adipose non-clipped). HOR and NOR categories could be separated for various fisheries during spring, summer and fall harvests.

Hatchery	Stock	Hatchery location	Hatchery broodstock	PBT tag rate	Spring harvest							Summer harvest						Fall harvest						Total												Grand total
					Commercial			Sport	Test		Wind R	Commercial		Sport	Tribal		Commercial		Sport	Tribal		Commercial			Sport		Test		Tribal							
					HOR	NOR	Unk		HOR	NOR		HOR	NOR		HOR	NOR	HOR	NOR		HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	Unk	HOR	NOR	HOR	NOR	HOR	NOR	Unk	
Carson National Fish Hatchery	Mixed origins (Snake and Mid-/Upper Columbia Rivers)	Columbia River	OtsCAR12	0.9000	16		1	75	12	1	12													16	0	1	75	0	12	1	12	0	0	117		
			OtsCAR13	0.9547			1	1															0	0	1	1	0	0	0	0	0	0	2			
Chief Joseph Hatchery	Integrated	Columbia River	OtsCJH13_sufa	0.8987										1		1						3	0	0	0	1	0	0	0	1	0	3	5			
Cleawater Fish Hatchery	South Fork Clearwater River	Snake River	OtsCLWH11S	0.9615	7			13	1														7	0	0	13	0	1	0	0	0	0	21			
			OtsCLWH12S	0.9202	3			29	5														3	0	0	29	0	5	0	0	0	0	37			
			OtsCLWH13S	0.9396	1			1	2															1	0	0	1	0	2	0	0	0	0	4		
Lookingglass Fish Hatchery	Catherine Creek	Snake River	OtsCTHW12S		2			2	1														2	0	0	2	0	1	0	0	0	0	5			
Dworshak National Fish Hatchery	Clearwater River	Snake River	OtsDWOR11S	0.9899	16			34	2														16	0	0	34	0	2	0	0	0	0	52			
			OtsDWOR12S	0.9926	37	1		136	28					1		1							37	1	0	137	0	28	0	1	0	0	204			
			OtsDWOR13S		2																			2	0	0	0	0	0	0	0	0	0	2		
Eastbank Fish Hatchery	Chelan Falls/Methow/Okanogan/Wenatchee	Columbia River	OtsEASTBK12	0.9619	5			4				1		8		7							6	0	0	12	0	0	0	7	0	0	25			
Lookingglass Fish Hatchery	Grande Ronde River	Snake River	OtsGRUW12S					1															0	0	0	1	0	0	0	0	0	0	1			
			OtsGRUW13S	0.9607		1		1																0	1	0	1	0	0	0	0	0	0	2		
Lookingglass Fish Hatchery	Imnaha River	Snake River	OtsIMNW11S	0.9443	4			2							1								4	0	0	2	0	0	0	1	0	0	7			
			OtsIMNW12S	0.9058	2			1																2	0	0	1	0	0	0	0	0	0	3		
			OtsIMNW13S	0.9789				3						2										0	0	0	5	0	0	0	0	0	0	5		
McCall Fish hatchery	Johnson Creek	Snake River	OtsJHNV11S	0.9747													1						0	0	0	0	0	0	0	0	1	0	1			
Klickitat Salmon Hathery	Klickitat River	Columbia River	OtsKH11S	0.8072	2			1						1									2	0	0	2	0	0	0	0	0	0	4			
			OtsKH12S	0.8300	4			5	3					1										4	0	0	6	0	3	0	0	0	0	13		
			OtsKH13S	0.9788	3			8						1										3	0	0	9	0	0	0	0	0	0	12		
Leavenworth National Fish Hatchery	Upper Columbia (mixed origins)	Columbia River	OtsLNFH13	0.8803	1																		1	0	0	0	0	0	0	0	0	0	1			
Lookingglass Fish Hatchery	Lookingglass Creek	Snake River	OtsLOOK11S	0.9747				3															0	0	0	3	0	0	0	0	0	0	3			
			OtsLOOK12S	0.9835	24			42	2			1		1		3	2							25	0	0	43	0	2	0	3	2	0	75		
			OtsLOOK13S	0.9502	1			1	1															1	0	0	1	0	1	0	0	0	0	3		
Lookingglass Fish Hatchery	Lostine River	Snake River	OtsLSTW11S	0.9153	2																		2	0	0	0	0	0	0	0	0	0	2			
			OtsLSTW12S	0.9677				1																0	0	0	1	0	0	0	0	0	0	1		
			OtsLSTW13S	0.9518				2						2											0	0	0	4	0	0	0	0	0	0	4	
Little White Salmon National Fish Hatchery	Little White Salmon River	Columbia River	OtsLWS13_sp	0.0000	1																		1	0	0	0	0	0	0	0	0	0	1			
			OtsLWS13_sufa	0.8763																			2	0	0	0	0	0	0	0	0	0	2	2		
Lyons Ferry Fish Hatchery	Mixed origins (Snake and Upper Columbia Rivers)	Snake River	OtsLYON11S_1														14						5	14	0	0	0	0	0	0	0	0	5	19		
			OtsLYON12S_1	0.9860											1		24						18	24	0	0	0	0	0	0	1	0	18	43		
			OtsLYON13S_1	0.9704																				1	0	0	0	0	0	0	0	0	0	1	1	
McCall Fish hatchery	South Fork Salmon River	Snake River	OtsMCCA11S	0.9904	3	1		7				3	1	2		2							6	2	0	9	0	0	0	2	0	0	19			
			OtsMCCA12S	0.9710	54	2		37				6	5	5		6	10							60	7	0	42	0	0	0	6	10	0	125		
Methow Fish Hatchery	Upper Methow and Twisp Rivers	Columbia River	OtsMETH12	0.9300	3			4		2													3	0	0	4	0	0	2	0	0	0	9			
Nex Perce Tribal Hatchery	Mixed origins (Snake and Clearwater River)	Snake River	OtsNPFH11S_1	0.8988													1						1	0	0	0	0	0	0	0	0	0	1			
			OtsNPFH12S_1	0.9937											2	1	14						12	14	0	0	0	0	0	0	2	1	12	29		
			OtsNPFH13S_1	0.9927													3						2	3	0	0	0	0	0	0	0	0	2	5		
Pahsimeroi Hatchery	Salmon River	Snake River	OtsPAHH11S	0.9662	3			2						1		2	2						3	0	0	3	0	0	0	2	2	0	10			
			OtsPAHH12S	0.9951	8			4	1					3										8	0	0	7	0	1	0	0	0	0	16		
			OtsPAHH13S		1			1						1			1							1	0	0	2	0	0	0	0	1	0	4		
Parkdale Fish Facility	Hood River	Columbia River	OtsPFF12	0.8900	14			5	1														14	0	0	5	0	1	0	0	0	0	20			
			OtsPFF13	0.6800										1										0	0	0	1	0	0	0	0	0	0	1		
Cleawater Fish Hatchery	Lochsa River (Powell Facility)	Snake River	OtsPOWP11S	0.9869	4			8		1													4	0	0	8	0	0	1	0	0	0	13			
			OtsPOWP12S	0.9866	9			46	8	3														9	0	0	46	0	8	3	0	0	0	66		
			OtsPOWP13S	0.9822	1			1	1															1	0	0	1	0	1	0	0	0</				

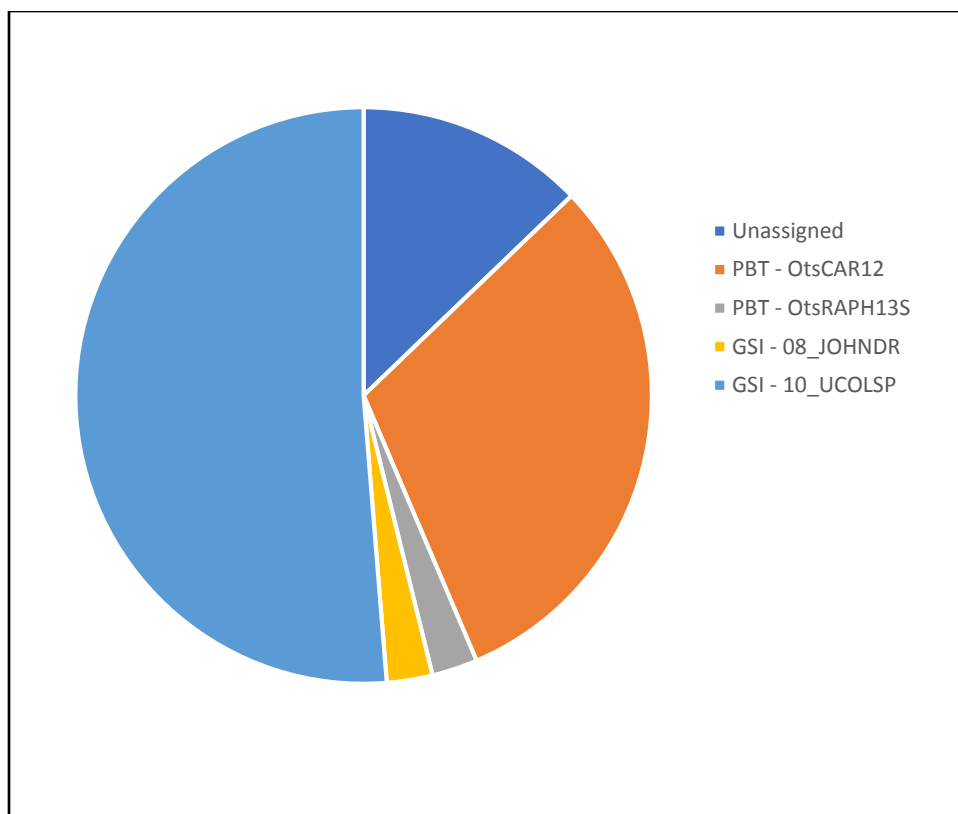


Figure 8: Genetic stock composition of the Wind River sport mark-selective harvest in 2016.

This analysis cannot currently quantify Carson Hatchery fish that are harvested in the bubble fishery, nor can it determine how many wild fish from other areas are handled in the fishery. Further, we cannot conclude whether changing the bubble fishery boundary has resulted in any change in impacts compared to previous years with the smaller bubble, because we did not sample in years prior to the bubble expansion.

Comparison of stock composition among spring management period Chinook salmon fisheries

Analysis of adipose-clipped Chinook salmon from multiple fishery mixtures in the spring management period (April to June 15th) identified relatively large proportions of individuals that assigned via PBT to Snake River hatcheries. Chinook salmon from Snake River hatcheries comprised 26-34% of fish harvested in Region B, and 37-50% of fish harvested in Region A from commercial, sport, and Test fisheries (Figure 9). These proportions are broadly consistent with the large proportion of hatchery origin Chinook salmon passing Bonneville Dam in 2016 that assigned via PBT to Snake River hatcheries (44%). As noted in the previous section, and unlike the other fisheries assessed, the Wind River sport fishery was not composed primarily of fish from Snake River hatcheries, but rather of fish that assigned via PBT to the Carson Hatchery on the Columbia River, and those that assigned via GSI to the 10_UCOLSP reporting group (Figure 9). PBT assignments to Columbia River hatcheries ranged from 10-19% and reflects the expansion of our PBT baseline for Columbia River hatchery broodstocks (i.e., in 2015 we were only able to assign 0-5.5% of harvest samples to Columbia River broodstocks). This is

consistent with the proportion of fish that assigned to Columbia River hatchery broodstocks (19%) (Table 12). In upcoming years, 5- year old fish will also be possible to assign to PBT baselines and additional age classes for specific hatcheries.

The majority of adipose-clipped Chinook salmon from fishery mixtures during the spring management period were assigned via GSI. Chinook salmon from the interior ocean type lineage (OT) comprised a small fraction (0-17%) of Chinook salmon taken in spring harvests, and was similar to the proportion passing Bonneville Dam (7%) during the spring management period (Figure 9). With the exception of the Wind River sport mark-selective harvest, Chinook salmon from the interior stream type lineage (ST) comprised 11-16% of harvest samples; slightly less than that observed at Bonneville Dam (22%), but a markedly smaller proportion than observed for these harvests in 2015 (19-45%). This may be attributable to the expansion of our PBT baseline in the Columbia River and the proportion of interior stream type Chinook that assigned to hatcheries which would have otherwise assigned via GSI to reporting groups that comprise the interior stream type lineage. As in 2015, we observed a decrease in the proportion of harvests comprised of the lower Columbia (LC) Chinook lineage up to Bonneville Dam (Figure 9). The proportion of LC lineage Chinook in Region B fisheries was 36-40%. This decreased to 5-14% for Region A fisheries, and to 0% at Bonneville Dam. The proportion of fish that could not be assigned using either PBT or GSI represented a small fraction of the harvest samples analyzed, and ranged from 4-8%; consistent with that observed at Bonneville Dam (7%) (Figure 9).

Limited sample sizes from adipose-intact commercial fisheries from Regions A (n=13) and B (n=6) restricted comparisons of stock composition to the test fishery (n=38). Analysis of the adipose-intact test fishery revealed that 13% of fish assigned via PBT to Snake River hatcheries (Figure 10); a lower fraction than that observed for adipose-clipped fisheries (26-31%; Figure 9) (as expected), but consistent with assignments for adipose-intact fish passing Bonneville Dam (17%). A greater proportion of adipose-intact fish in the test fishery assigned to Columbia River hatcheries (13%) relative to those at Bonneville Dam (4%). The LC lineage comprised 21% of the test fishery, but only 1% of the fish encountered passing Bonneville Dam. The OT lineage was observed at Bonneville Dam, but not in the test fishery. The ST lineage comprised the greatest proportion of adipose-intact test fishery (29%), and approximated that encountered at Bonneville Dam (35%) during the spring management period (Figure 10). Interestingly, 24% of adipose-intact fish in the test fishery and 23% of adipose intact fish at Bonneville Dam could not be assigned either via PBT or GSI with our assignment thresholds.

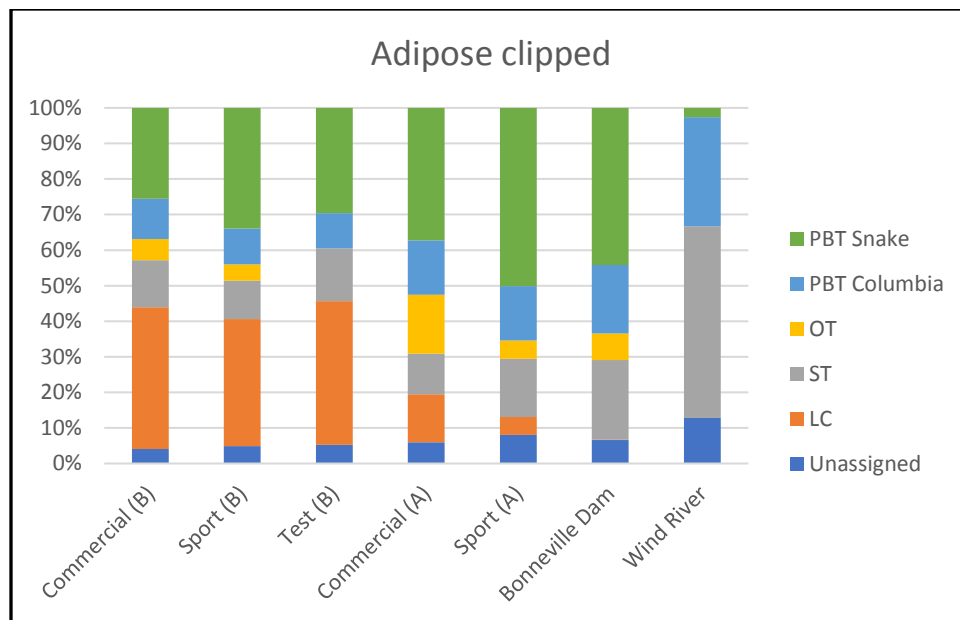


Figure 9: Stock composition of spring management period adipose-clipped Chinook salmon harvest mixtures. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

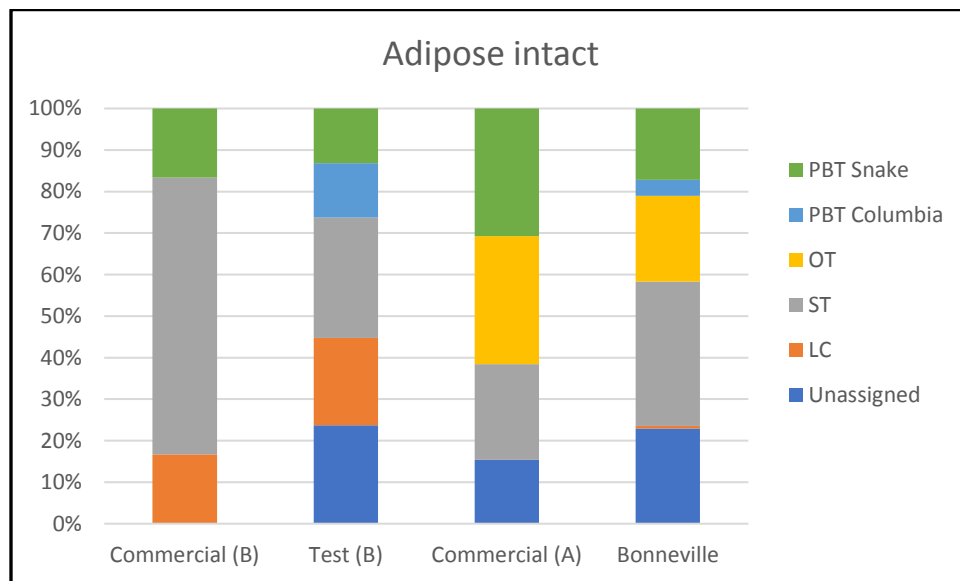


Figure 10: Stock composition of spring management period adipose-intact Chinook salmon harvest mixtures. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

Comparison of percent stock composition of upriver spring Chinook salmon stocks (ST) among summer-management period Chinook salmon fisheries

Analysis of Chinook salmon fisheries in the summer management period (June 16 – August 1) addressed the following objectives: 1) estimate stock composition for the mark selective sport fishery and commercial fishery below Bonneville Dam, 2) compare stock composition of adipose-clipped versus adipose-intact fish from the commercial harvest below Bonneville Dam, 3) characterize temporal changes in stock composition across the season. While jack Chinook salmon are not harvested at high rates in fisheries and there are no specific harvest limits for them, jack Chinook salmon if sampled could have been incorporated into this analysis.

We observed similar stock compositions for adipose-clipped Chinook salmon taken in Lower Columbia River sport and commercial fisheries from Region B (Figure 11). However, Chinook stocks from the OT lineage comprised a greater proportion of the sport fishery (59%) than the commercial harvest (39%). While a similar proportion of adipose-clipped fish in commercial and sport fisheries from Region B assigned via PBT, a greater proportion of the sport harvest was assigned to Columbia River hatcheries (9%) than the commercial harvest (6%), and a greater proportion of the commercial harvest assigned to Snake River hatcheries (14%) than the sport harvest (6%) (Figure 11). A similar comparison between adipose-clipped sport and commercial fisheries in Region A was restricted by small sample size for the Region B commercial harvest (n=4).

Although comparisons between adipose-clipped commercial fisheries in Region A and B was restricted by a small sample size for the Region B commercial harvest (n=4), we were able draw comparisons between adipose-clipped sport fisheries with larger sample sizes. While a greater proportion of the LC lineage was observed in the adipose-clipped sport harvest in Region B (18%) than in Region A (3%), we detected a greater proportion of the OT lineage in Region A (71%) vs. Region B (59%) (Figure 11). A similar proportion of fish in Region B (9%) assigned via PBT to Columbia River hatcheries vs. Region A (7%). However, a greater proportion of fish in Region A (11%) assigned via PBT to Snake River hatcheries vs. those from Region A (6%). We observed no appreciable difference between these fisheries in the relative proportion of fish that assigned to stocks from the ST lineage (Figure 11).

Despite broad similarities in the stock composition of adipose intact commercial fisheries from Regions A and B, we also detected some differences. A greater proportion of fish in Region B (24%) assigned to the LC lineage than those from Region A (12%), while a greater proportion of Region A fish (79%) assigned to the OT lineage than those in Region B (68%) (Figure 11).

We observed notable differences in the stock composition of adipose-clipped and adipose-intact Chinook salmon taken in the commercial fishery in Region B. As expected, a greater proportion of adipose-clipped fish assigned to Snake River hatcheries (14%) and Columbia River hatcheries (6%) than adipose-intact fish (2% and 0%, respectively). We also observed that a smaller proportion of the adipose-clipped commercial harvest was comprised of

the OT lineage (39%) than the adipose-intact harvest (68%) (Figure 11). A greater proportion of adipose-clipped fish (35%) assigned to the LC lineage than adipose-intact fish (24%). We observed no appreciable difference between the adipose-clipped and adipose-intact commercial harvest in Region B in the relative proportion of fish that assigned to stocks from the ST lineage (Figure 11).

We observed differences in the stock composition of adipose-intact and adipose-clipped fish taken in the Zone 6 tribal harvests. As observed in other comparisons of adipose-clipped vs. adipose-intact fisheries, a greater proportion of adipose-clipped fish assigned to Columbia River hatcheries (9%) vs. adipose-intact fish (2%) (Figure 11). We also detected a greater proportion of adipose-intact fish assigned to the OT lineage (81%) than adipose-clipped fish (76%). Across all comparisons, the proportion of fish that could not be assigned using either PBT or GSI was low, and ranged from 3-8% (Figure 11).

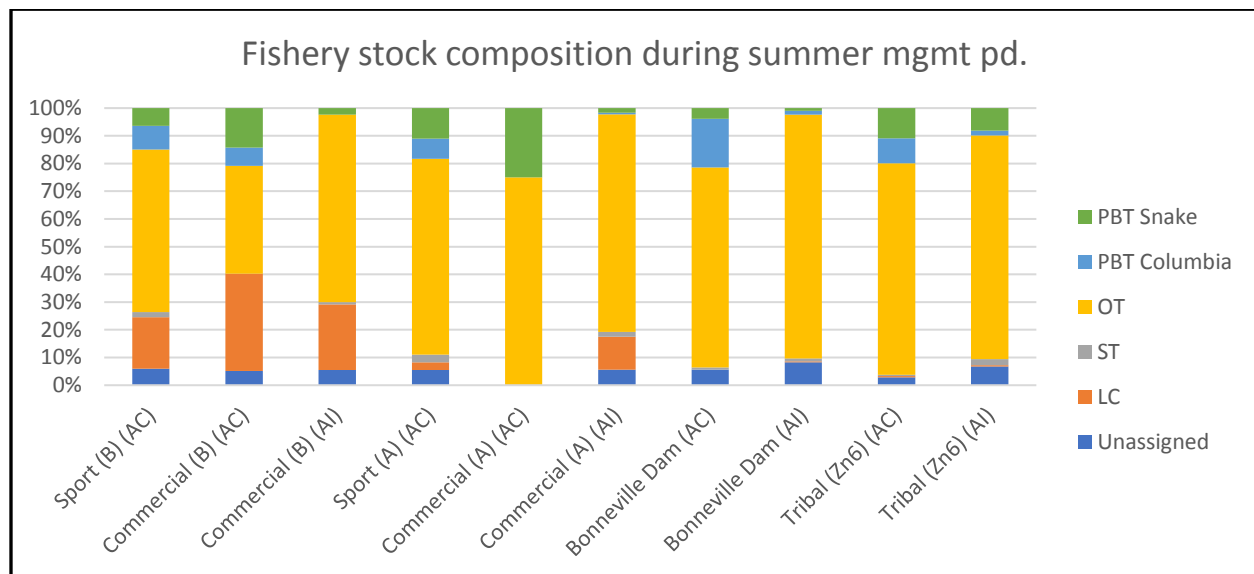


Figure 11: Stock composition of summer management period Chinook salmon fisheries and Bonneville Dam. 'AC' is adipose-clipped; 'AI' is adipose intact. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

We attempted to compare changes in the percent stock composition of the stream-type lineage of adipose-clipped vs. adipose-intact Chinook salmon over the course of the summer management period in the lower Columbia River commercial fishery relative to that passing Bonneville Dam. However, we were limited by small sample sizes that restricted our interpretation. We detected declines in the proportion of ST lineage Chinook salmon, for both adipose-clipped and adipose-intact fish at Bonneville Dam over the summer management period (Figure 12). However, there was a modest increase in the proportion of the ST lineage for adipose-clipped fish at Bonneville Dam in statistical week 27. We also detected a decline for

adipose-intact fish from the commercial fishery, but only detected the ST lineage during statistical week 25 for adipose-clipped fish, and this limited our interpretation. As in previous years, meaningful comparisons are made challenging by the absence of data continuity over the time series owing to fisheries closures and cessation of sampling at the Bonneville AFF in response to elevated water temperatures.

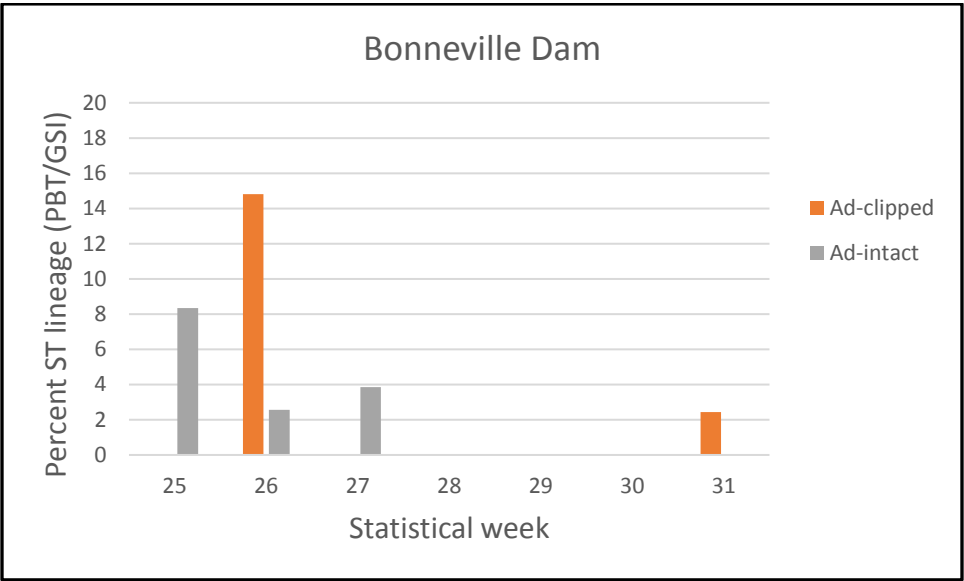
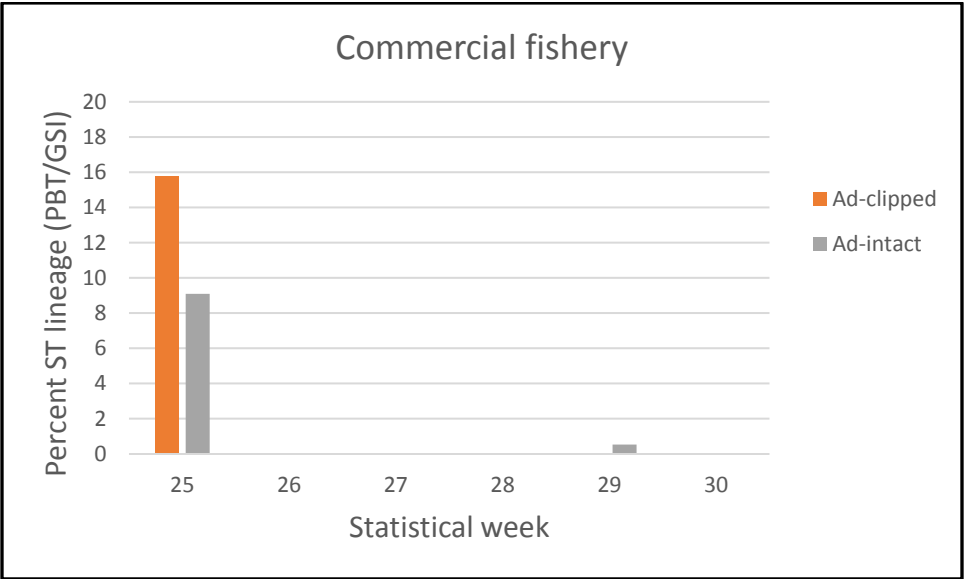


Figure 12: Temporal patterns of the percent of Chinook salmon ST lineage in adipose-clipped and adipose intact mixture samples from the lower Columbia River commercial fishery (top panel) and Bonneville Dam (bottom panel) during the summer management period (June 16-August 31).

1127 *Comparison of stock composition among sockeye salmon fisheries*

1128 Sockeye salmon were sampled from the lower Columbia River below Bonneville Dam in
1129 the lower river sport and commercial fishery and above Bonneville Dam in the Zone 6 tribal
1130 fishery, and were assigned to five Columbia River genetic stocks (Table 13). The Zone 6 sport
1131 fishery was not sampled in 2016. Samples from the lower Columbia River commercial harvest
1132 were only obtained during statistical week 25, and we did not observe any assignment to the
1133 Redfish Lake stock from this fishery in 2016. Low sample numbers of *O. nerka* make it difficult
1134 to estimate narrow confidence intervals for abundance estimates of the Redfish Lake, Lake Billy
1135 Chinook, and Wallowa Lake stocks (Table 14).

1136
1137 The timing of the sockeye salmon fisheries may influence the harvested proportion of
1138 each stock, and is consistent with run-timing distributions observed at Bonneville Dam;
1139 particularly that the Okanogan and Wenatchee stocks had nearly identical run-timing
1140 distributions in 2016, but migrated passed Bonneville Dam earlier than other stocks. The
1141 Redfish Lake stock was only represented by 15 fish in the Zone 6 tribal fishery sample (Table
1142 13) making run-timing estimates imprecise for this stock. Of the 15 Redfish Lake fish identified
1143 with GSI, the largest number (n=5) were sampled in weeks 28 and 29 (Figure 13). The highest
1144 proportions of Okanogan stock (64%) and Wenatchee stock (55%) in the Zone 6 tribal fishery
1145 occurred during statistical weeks 25 and 31, respectively (Figure 13). Notable difference in
1146 stock proportions between Bonneville Dam and the Zone 6 tribal harvest were observed for the
1147 Okanogan stock (58% vs. 70%) and for the Wenatchee stock (41% vs. 29%) in the harvest vs.
1148 Bonneville Dam mixture samples, respectively (Table 14).

1149 Table 13: Summary of sample sizes and stock assignments for sockeye salmon fisheries by weekly strata.

Fishery	Stock	Statistical week						
		25	26	27	28	29	30	31
Commercial	Okanogan	31	0	0	0	0	0	0
	Wenatchee	26	0	0	0	0	0	0
Sport	Okanogan	10	30	12	5	3	4	1
	Redfish Lake	0	0	1	0	0	0	0
	Wenatchee	4	17	5	0	1	2	0
Zone 6	Okanogan	190	405	325	125	52	13	18
	Redfish Lake	1	1	5	5	1	1	1
	Wenatchee	108	287	223	80	47	12	23
	Lake Billy Chinook	0	0	1	1	1	0	0
	Lake Wallowa	0	0	0	2	0	0	0
	Unassigned	1	3	2	0	1	0	2

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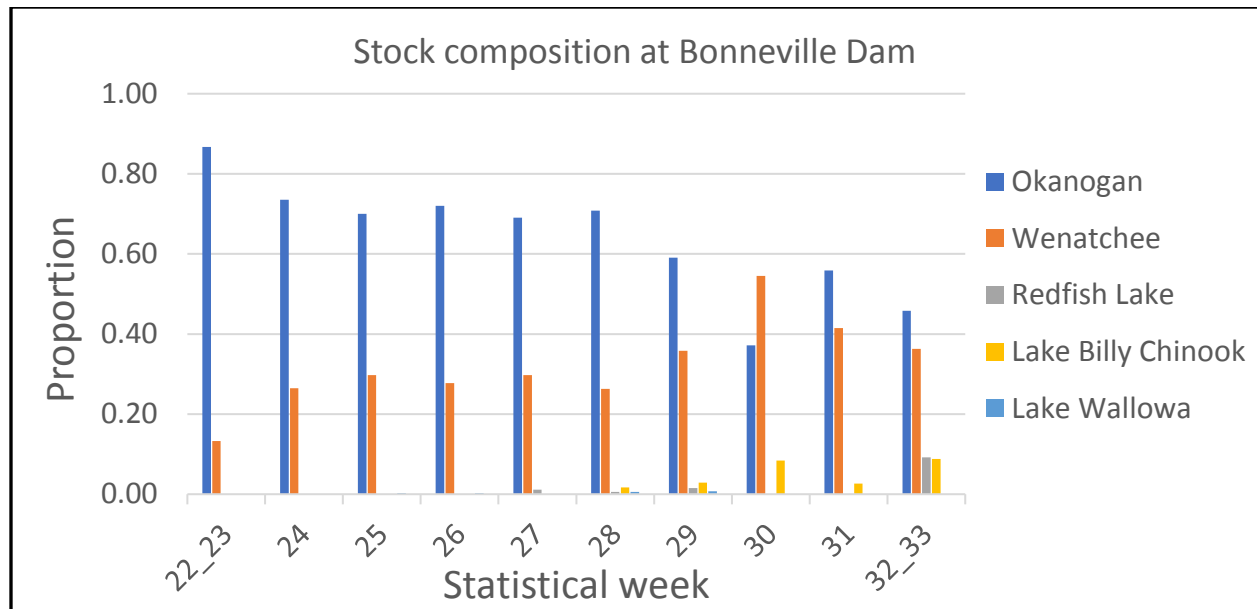
1151 Table 14: Comparison of stock-specific abundance and percent composition among sockeye salmon fisheries. The mean stock abundance estimate is provided for each fishery
1152 harvest and includes 95% confidence intervals.

Mixture source	Mean (95% C.I.)					Stock proportion				
	Okanagan	Wenatchee	Redfish Lake	Lake Billy Chinook	Lake Wallowa	Okanagan	Wenatchee	Redfish Lake	Lake Billy Chinook	Lake Wallowa
Commercial	193 (150 – 237)	163 (119 – 206)	-	-	-	54.08%	45.92%	-	-	-
Sport	508 (439 – 572)	228 (164 – 298)	18 (0 – 23)	-	-	68.29%	30.67%	1.04%	-	-
Zone 6	9,684 (9319 – 10058)	6,757 (6,391 – 7,127)	132 (71 – 201)	30 (0 – 70)	14 (0 – 201)	58.28%	40.67%	0.79%	0.18%	0.09%
Total Harvest	10,419 (10,042 – 10,800)	7,119 (6,746 – 7,487)	136 (77 – 213)	25 (0 – 60)	17 (0 – 43)	58.81%	40.19%	0.77%	0.14%	0.09%
Bonneville Dam*	226,095 (212,059 – 228,241)	82,331 (94,224 – 99,142)	1,112 (142 – 2,025)	1,073 (276 – 1,381)	799 (0 – 1,756)	69.93%	29.14%	0.34%	0.33%	0.25%

1153 * Bonneville Dam abundance estimates shown here differ from those in Table 3 (Chp 4) since they only include the interval of weeks that coincide with sockeye salmon fisheries
1154 (statistical weeks 25-31).

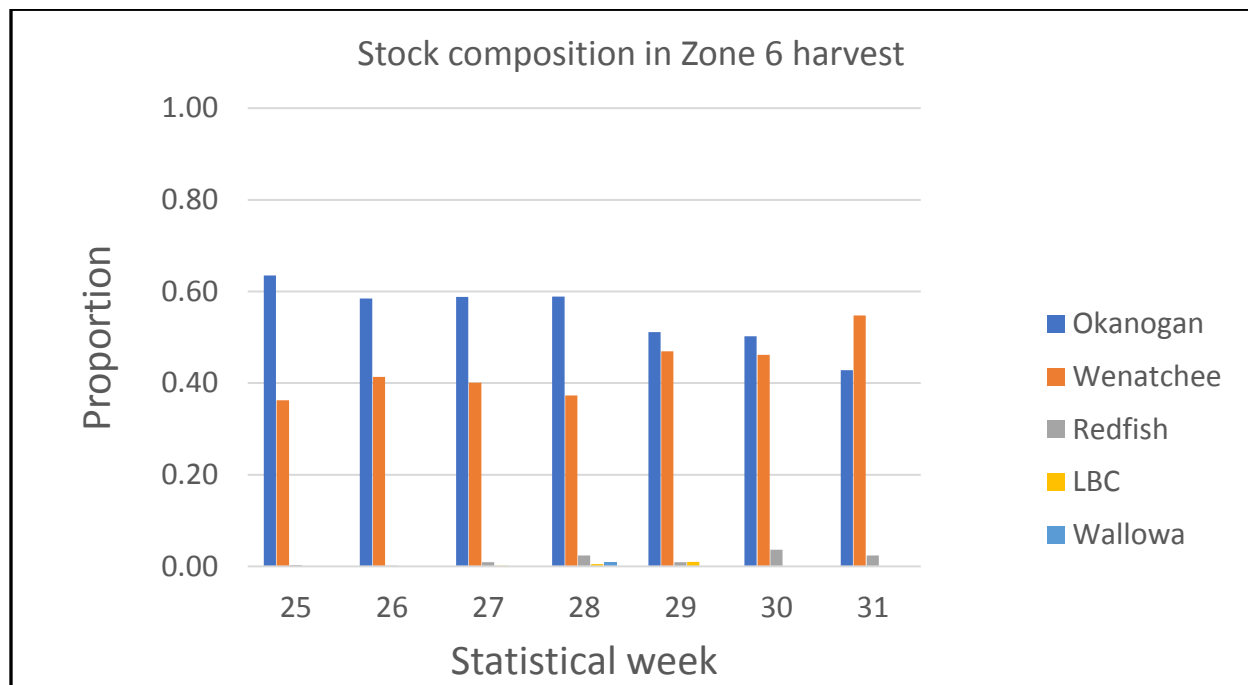
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1160 Figure 13: Stock composition of sockeye salmon at Bonneville Dam (top panel) and in the Zone
1161 6 tribal harvest (bottom panel) across weekly strata.

Discussion

Management implications

This study utilized both genetic stock identification (GSI) and parentage based tagging (PBT) in combination to estimate stock composition of mainstem Columbia River Chinook salmon and sockeye fisheries. This is the fourth year in which we were able to assign all three major age classes of spring Chinook from Snake River hatcheries and the first year in which we could assign 3- and 4-year old fish to Columbia River hatcheries as a consequence of our expanded PBT baseline. Ongoing expansion of the PBT baseline will allow us the ability to assign Snake River hatchery jacks of the fall-run Chinook salmon as well as all other hatchery jacks originating above Bonneville Dam (migration year 2017), and so future years of analyses will include more emphasis on fall-run harvest. Expansion of the PBT baseline to include not only hatcheries of Chinook salmon and steelhead above Bonneville Dam, but also hatcheries throughout the range of these species could eventually lead to replacing the coded wire tag program for monitoring of in-river harvest stock composition of these species if increases in funding were available and fishery managers thought it were needed. This report includes the fourth genetic analysis on sockeye salmon harvest. Our results demonstrated differences in stock composition of the sockeye salmon harvest as compared to the total run estimated at Bonneville Dam, but there are questions about the validity of the estimates especially at Bonneville given the potential for sampling error around rare stocks like Snake R. sockeye salmon. We will continue to perform GSI on sockeye salmon harvest in the future to gain further insight into these patterns.

One higher level management question was possible to address in this section:

1) Harvest RM&E: F&W Program Management Question: What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?

The in-river estimates of stock composition, stock-specific abundance, escapement, catch, and age distribution were addressed for part of the treaty mainstem spring-management period fisheries Chinook salmon harvests above Bonneville Dam along with fisheries below Bonneville Dam, the non-treaty summer-management fisheries below Bonneville Dam which are a portion of the total non-treaty summer fisheries, and the mainstem treaty sockeye salmon harvests above and non-treaty harvest below Bonneville Dam. For the spring management period of Chinook salmon, we continue to observe a spatial pattern for the stock composition of lower Columbia River stocks which appear more abundant downstream from the Willamette River mouth as compared to upstream of this point which is consistent with a long history of CWT data. We observed differences in the composition of hatchery stocks represented in spring vs. summer management period harvest of Chinook salmon, and run-timing plays an important role in this difference (i.e., late-running stocks appear more abundant among the upriver spring-type lineage that are caught in the summer management period). This pattern is consistent when compared to known origin PIT tagged adult and jack fish tagged as juveniles. Known origin adult age upriver spring and Snake River spring Chinook salmon are almost all past Bonneville by June 15 in most years. However, specific conclusions relative to the harvest impacts on spring run Chinook salmon cannot be made from this genetic analysis as jacks are included in the current study.

1208
1209 This study also addressed some issues that relate to mark-selective fisheries. For
1210 example, we examined stock composition of the Wind River mark-selective spring-run Chinook
1211 salmon sport fishery and provided context of stock composition observed among other fisheries
1212 during this management period. The number of PBT-assigned Snake R. fish that were harvested
1213 in the Wind River sport fishery was less than all other fishery samples we analyzed, including
1214 the adipose-intact samples from Bonneville Dam and tribal harvests during the same set of
1215 weeks. For the 2016 analyses, our Columbia River PBT baseline should be sufficient to assign
1216 4-year-old fish from the Wind River sport fishery to the Carson hatchery. It was not possible to
1217 conclude whether or not the Wind River sport fishery harvest composition has significantly
1218 changed through time given this boundary change.

1219
1220 The sockeye salmon tribal fishery is managed in a way that attempts to harvest as many
1221 harvestable sockeye salmon as possible under the allowed harvest rate schedule in the U.S. v.
1222 Oregon Management Agreement. This 2015 2016 year of analysis of the sockeye salmon
1223 harvest corroborates harvest analyses from previous years, which suggested there may be some
1224 over representation of the Wenatchee sockeye stocks in the Zone 6 harvest as compared to the
1225 stock proportions that are present at Bonneville Dam. The results for Snake River sockeye
1226 salmon are dependent upon representative sampling at Bonneville Dam, but low sample rate and
1227 the rarity of this stock led to uncertainty and high variation around estimates of Snake River
1228 sockeye salmon from Bonneville Dam. Sampling protocols at Bonneville Dam may have higher
1229 representation of young fish as compared to harvest mixtures. Timing of the fishery may also
1230 influence the proportion of each stock, and is consistent with run-timing distributions we
1231 observed in previous reports; the Wenatchee stock has relatively early run-timing but the timing
1232 of the Snake River stock is uncertain due to inconsistent results between PIT-tag and GSI
1233 methods. Future analysis will be needed to examine these patterns for consistency and delve into
1234 explanations.

References

- Anderson, E. C., R. S. Waples, and S. T. Kalinowski. 2008. An improved method for estimating the accuracy of genetic stock identification. *Canadian Journal of Fisheries and Aquatic Sciences* 65:1475-1486.
- Anderson E. C. 2010. Computational algorithms and user-friendly software for parentage-based tagging of Pacific salmonids. Final report submitted to the Pacific Salmon Commission's Chinook Technical Committee (US Section). 46 p.
<http://swfsc.noaa.gov/textblock.aspx?Division=FED&ParentMenuId=54&id=16021>.
- Beacham, T. D., J. R. Candy, K. L. Jonsen, J. Supernault, M. Wetklo, L. T. Deng, K. M. Miller, R. E. Withler, and N. Varnavskaya. 2006. Estimation of stock composition and individual identification of Chinook salmon across the Pacific Rim by use of microsatellite variation. *Transactions of the American Fisheries Society* 135(4):861-888.
- Beacham, T. D., B. McIntosh, and C.G. Wallace. 2011. A comparison of polymorphism of genetic markers and population sample sizes required for mixed-stock analysis of sockeye salmon (*Oncorhynchus nerka*) in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, 2011, 68(3): 550-562.
- Blankenship, S. M., M. R. Campbell, J. E. Hess, M. A. Hess, T. W. Kassler, C. C. Kozfkay, A. P. Matala, S. R. Narum, M. M. Paquin, M. P. Small, J. J. Stephenson, K. I. Warheit and P. Moran. 2011. Major Lineages and Metapopulations in Columbia River *Oncorhynchus mykiss* Are Structured by Dynamic Landscape Features and Environments. *T. Am. Fish. Soc.* 140:665–684.
- Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics* 5: 164-166.
- Hess, J. E., A. P. Matala, and S. R. Narum. 2011. Comparison of SNP and microsatellite markers for application of genetic stock identification for Chinook salmon in the Columbia River Basin. *Molecular Ecology Resources* 11 (Suppl. 1):1–13.
- Hess, J. E. and S. R. Narum. 2011. SNP loci correlated with run-timing in adult Chinook salmon from the Columbia River Basin. *Transactions of the American Fisheries Society* 140(3):855-864
- Hess, J.E., N.R. Campbell, A.P. Matala, S.R. Narum. 2012. 2011 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Hess, J.E., N.R. Campbell, A.P. Matala, S.R. Narum. 2013. 2012 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Hess, J.E., N.R. Campbell, A.P. Matala, D.J. Hasselman, and S.R. Narum. 2015. 2014 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.

- Hess, J.E., J.M. Whiteaker, J.K. Fryer, and S.R. Narum. 2014. Monitoring stock-specific abundance, run timing, and straying of Chinook salmon in the Columbia River using genetic stock identification (GSI). *North American Journal of Fisheries Management* 34: 184-201.
- Hess, J.E., M.W. Ackerman, Jeffrey K. Fryer, D.J. Hasselman, C.A. Steele, J.J. Stephenson, J.M. Whiteaker, and S.R. Narum. 2016. Differential adult migration-timing and stock-specific abundance of steelhead in mixed stock assemblages. *ICES Journal of Marine Science* 73:2606-2615.
- Narum, S. R., T. L. Schultz, D. M. Van Doornik and D. Teel. 2008. Localized genetic structure persists in wild populations of Chinook salmon in the John Day River despite gene flow from outside sources. *Transactions of the American Fisheries Society* 137:1650-1656.
- Narum, S. R., J.E. Hess, and A.P. Matala. 2010. Examining genetic lineages of Chinook salmon in the Columbia River Basin. *Transactions of the American Fisheries Society* 139:1465-1477.
- Nei, M. 1972. Genetic distance between populations. *The American Naturalist* 106:283-292.
- Piry, S., A. Alapetite, J.M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GeneClass2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* 95: 536-539.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155(2): 945–959.
- Rannala, B. and J.L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences* 94:9197-9201.
- Saitou, N., and M. Nei .1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425
- Seeb, L. W., A. Antonovich, M. A. Banks, T. D. Beacham, M. R. Bellinger, S. M. Blankenship, M. R. Campbell, N. A. Decovich, J. C. Garza, C. M. Guthrie, T. A. Lundrigan, P. Morgan, S. R. Narum, J. J. Stephenson, K. J. Supernault, D. J. Teel, W. D. Templin, J. K. Wenburg, S. F. Young, and C. T. Smith. 2007. Development of a standardized DNA database for Chinook salmon. *Fisheries* 32(11):540-552.
- Shaklee, J. B., T. D. Beacham, L. Seeb, and B. A. White. 1999. Managing fisheries using genetic data: case studies form four species of Pacific salmon. *Fisheries Research* 43(1-3)45-78.
- Steele CA, Campbell MR, Ackerman M, McCane J, Hess MA, Campbell N, Narum SR. 2011. Parentage Based Tagging of Snake River hatchery steelhead and Chinook salmon. Bonneville Power Administration. Annual Progress Report, Project number 2010-031-00.

1327 [https://research.idfg.idaho.gov/Fisheries%20Research%20Reports/Res11-](https://research.idfg.idaho.gov/Fisheries%20Research%20Reports/Res11-111Steele2010%20Parentage%20Based%20Tagging%20Snake%20River%20Steelhead%20Salmon.pdf)
1328 [111Steele2010%20Parentage%20Based%20Tagging%20Snake%20River%20Steelhead](https://research.idfg.idaho.gov/Fisheries%20Research%20Reports/Res11-111Steele2010%20Parentage%20Based%20Tagging%20Snake%20River%20Steelhead%20Salmon.pdf)
1329 [%20Salmon.pdf](https://research.idfg.idaho.gov/Fisheries%20Research%20Reports/Res11-111Steele2010%20Parentage%20Based%20Tagging%20Snake%20River%20Steelhead%20Salmon.pdf)
1330
1331 Waples, R.S., Teel, D.J., Myers, J.M., Marshall, A.R., 2004. Life-history divergence in
1332 Chinook salmon: historical contingency and parallel evolution. *Evolution*, 58(2):
1333 386–403.
1334
1335 Winans GA, Paquin MM, Van Doornik DM (2004) Genetic stock identification of steelhead in
1336 the Columbia River basin: An evaluation of different molecular markers. *North American*
1337 *Journal of Fisheries Management* 24, 672-685.

Section 4: Characterization of Chinook salmon, sockeye salmon, and steelhead run-timing and abundance at Bonneville Dam

Introduction

The Columbia River Basin supports ESA listed natural-origin stocks of Chinook salmon and steelhead as well as hatchery supplemented populations. Both Chinook salmon and steelhead have been declining in the Columbia River Basin for several reasons including climate change, habitat degradation, hydropower, hatchery practices, and over-harvesting. Along with abundance estimates, basic information related to the way in which stocks of salmonids are spatiotemporally distributed are needed by fisheries managers to achieve sustainable fisheries.

As evident from the genetic stock identification (GSI and PBT) analyses of Chinook and sockeye salmon fisheries harvests in Section 3, certain stocks seem to have strong spatial and temporal associations. However, because the type of fishing gear, harvest regulations, and the locations targeted varies considerably among fisheries, samples from a representative mixture of all hatchery- and natural-origin stocks at a fixed location is expected to more accurately estimate relative abundance and characterize run-timing distributions of stocks. One potentially ideal fixed location for such sampling is Bonneville Dam, but trapping limitations at this location continue to pose a major challenge for sampling. In addition to information on abundance and run-timing, biological data including fork length and age can be examined with estimated stock of origin to characterize life history differences among stocks. This type of examination is especially important for steelhead, which has been managed using two life-history categories (i.e., A- and B-run). These life-history categories have been observed to be differentially characterized by run-timing at Bonneville Dam (e.g., B-run typically arrives after Aug. 25th), fork length (e.g., by definition, B-run fish are >78 cm), and ocean age (e.g., most B-run fish tend to spend 2 or more years in saltwater) and all of these types of data have been collected for steelhead in this study.

Project objectives and higher level harvest management questions

Here we analyze fish across the entire run of steelhead, Chinook and sockeye salmon from April to October to estimate temporally stratified proportions of stocks and extrapolate abundance using a daily census that is conducted at the Bonneville Dam fish counting window. We examine steelhead, Chinook salmon, and Sockeye salmon using sets of species-specific SNP assays for up to 379 loci per species. Although there are some methodological differences among these species-specific applications (e.g., different temporal strata), the general approach to estimating abundance and characterizing run-timing distributions was applied consistently across species. For all three species, we have demonstrated that these genetic baselines are generally accurate for assigning fish of unknown origin, but the genetic similarity of some stocks requires large reporting groups comprised of broad geographic areas (i.e., mid-Columbia R. and lower Snake R. for spring Chinook salmon). Since Bonneville Dam is the most downstream dam on the Columbia River, the mixture samples obtained here represent the majority of upriver/interior Columbia River Basin stocks. This ongoing study offers a rare opportunity to monitor populations of multiple species of salmonids from a broad geographic range over several years. This long-term study will allow us to characterize trends in run timing and abundance of steelhead, Chinook and sockeye salmon and provide this data to fisheries managers. However,

the genetic stock units ('reporting groups') are not the same units that groups of fish are currently managed for due to levels of genetic differentiation that can be detected among baseline stocks (see results under Objective 2 for details). Thus, fisheries managers continue to explore how to best incorporate genetic monitoring results with more traditional monitoring/tagging programs.

Harvest RM&E: F&W Program Management Questions:

- i) **What are the status and trend of adult productivity of fish populations?**
- ii) **What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?**

Analysis of the 2011 dataset by Hess et al. (2012) was the first year we were able to apply Parentage Based Tagging (PBT) to assign a portion of Snake River hatchery-origin spring-run Chinook salmon and summer-run steelhead back to their hatchery parents (Steele et al. 2011). This powerful genetic tool provides the opportunity to obtain additional types of data including accurate age of fish, quantification of the number of non-adipose clipped hatchery-origin fish, and precise assignments of fish to their hatchery broodstock (Steele et al. 2013). The ability of PBT to assign fish to their hatchery broodstock has been shown to be equally accurate as traditional tags (e.g., CWTs; Steele et al. 2013), and PBT provides assignments to specific hatchery broodstocks rather than larger reporting groups used in GSI methods. However, these tools can provide the greatest benefit when applied in combination, as GSI has the ability to provide information on natural-origin fish throughout the Columbia River basin, while PBT is most effective for hatchery-origin fish. The current PBT baseline was recently expanded beyond Snake River hatcheries to include others above Bonneville Dam. However, this effort is ongoing, and while hatcheries continue to be added to our PBT baseline annually, GSI remains a necessary tool for both hatchery- and natural-origin fish that originate from outside the Snake River basin. This report is the fourth year in which all major age classes of steelhead (i.e. 1-, 2-, and 3- ocean ages) and Chinook salmon (3-, 4-, and 5-year olds) can be assigned using the PBT baseline of Snake River hatcheries, and the second year in which these can be assigned to some Columbia River hatcheries. This study integrates PBT and GSI results to provide the greatest amount of stock-specific information available for hatchery- and natural-origin steelhead and Chinook salmon passing Bonneville Dam.

Time line for completion of objectives

Objectives will be ongoing and GSI results updated each year for analyses of salmon and steelhead throughout the accords-funding. As new genetic techniques are developed they will be applied to this project and results will be compared between years to determine the extent of improvements.

Methods

Sample Collection

Tissue samples were obtained from adult steelhead (n=1485), Chinook (n = 3259) and sockeye salmon (n=812) adults in 2016 during migration runs at Bonneville Dam. This sampling effort is covered under the 2008-2017 US vs. Oregon harvest biological opinion for sampling at Bonneville Dam.

Sampling for Chinook salmon at Bonneville Dam began during statistical week 16 (April 15, 2016). Sampling occurred at the Adult Fish Facility (AFF) located on the northern end of Bonneville Dam. Fish were sampled 4–5 d per statistical week (except when reduced due to restrictions on trap use or low run size at the beginning and end of the run) and for 4–6 h per day. A picket weir was used to divert migrating fish ascending the Washington shore fish ladder into the AFF collection pool. An attraction flow was used to draw fish through a false weir where they were selected for sampling. After sampling was completed and fish recovered from the anesthetic, they were returned to the Washington shore fish ladder above the picket weir. Just 2.0% of the total Spring management period (i.e., January 1-June 15) adult Chinook salmon count had passed Bonneville by the sampling start date (April 15). While samples were taken from the majority of the total spring Chinook salmon run, some early timed stocks may be slightly under-estimated in the results. Restrictions imposed by USACE and NMFS on sampling at the Bonneville AFF result in sample rates for Chinook, sockeye, and steelhead that are often low. The average sample rate (weeks 16–25) for the adult and jack spring Chinook run in 2016 was 0.89%, whereas the average sample rate for adult and jack summer Chinook was 0.69% (Table 1).

Based on numbers of fish collected, samples were pooled into weekly strata for Chinook (Table 1), monthly strata for steelhead (Table 2), or a combination thereof for sockeye salmon (Table 3) spanning the majority of the run-year from April to October. We followed a similar protocol as the Monitoring Methods [Protocol "Snake River steelhead and Chinook salmon stock composition estimates \(2010-026-00\) v1.0"](#).

Molecular markers

Expanded panels of genetic markers for steelhead (379 SNPs) and sockeye salmon (382 SNPs) are provided in Section 1, while the GT-seq panel of 298 SNPs for Chinook salmon is provided in Hasselman et al. (2017).

Statistical analyses

Snake River Chinook salmon and steelhead were analyzed for [Parentage assignments using SNPPIT software v1.0](#) (ID: 1341) (Published). The program ONCOR was used to estimate the most likely population-of-origin for the sockeye salmon samples. Individuals were assigned using a ‘best estimate’ approach [Assigning individual samples using Individual Assignment \(IA\) genetic methods v1.0](#) (ID: 1334) (Published). We used GSIsm for [Mixture modeling to estimate stock proportions v1.0](#) (ID: 1333) (Published) to estimate stock composition of Bonneville Dam mixture strata for Chinook salmon and steelhead. Additional detail regarding the specific application to Bonneville Dam are published in Hess et al. (2013).

1459 Table 1: Sample numbers by weekly strata for Chinook salmon that were DNA sampled or
 1460 tallied for abundance at Bonneville Dam in 2016.

		Statistical week	Bonneville dam fish window count	Sample (N)					
				Clipped		Non-clipped		Sample	
				GSI	PBT	GSI	PBT	Total	Rate
Management period	Spring	16	2837	3	5	0	0	8	0.28%
		17	9712	26	63	17	4	110	1.13%
		18	20859	24	69	21	7	121	0.58%
		19	58037	54	182	44	17	297	0.51%
		20	21512	46	79	35	15	175	0.81%
		21	18396	59	121	79	27	286	1.55%
		22	11112	62	75	69	19	225	2.02%
		23	12938	38	28	46	3	115	0.89%
		24	17270	39	27	58	12	136	0.79%
		25sp	12490	13	5	17	3	38	0.30%
	Summer	25su	9311	17	6	24	2	49	0.53%
		26	22170	21	8	39	0	68	0.31%
		27	18839	29	6	53	1	89	0.47%
		28	14912	18	11	47	1	77	0.52%
		29	10784	32	9	39	1	81	0.75%
		30	8779	30	3	61	2	96	1.09%
		31	6609	37	7	32	0	76	1.15%
	Fall	32	5665	10	4	29	6	49	0.86%
		33	10274	12	5	39	14	70	0.68%
		34	23675	12	17	35	16	80	0.34%
		35	59099	42	11	71	13	137	0.23%
		36	91960	36	16	86	26	164	0.18%
		37	124538	30	8	107	18	163	0.13%
		38	96709	58	13	147	17	235	0.24%
		39	43015	10	5	86	9	110	0.26%
		40	19350	9	5	91	12	117	0.60%
		41	10266	5	2	47	5	59	0.57%
		42	4845	3	2	19	4	28	0.58%
		Total	765963	775	792	1438	254	3259	0.43%

1461 Note: Statistical week 16 is 4/10/16–4/16/16 and 42 is 10/9/16–10/15/16. ‘Fish count’ is based
 1462 on tallies of Chinook salmon adults and jacks provided by the Fish Passage Center
 1463 (<http://www.fpc.org>) observed by the Corps of Engineers at their fish counting window. The total
 1464 sum of all samples for a given week was used to calculate sample rate. The management periods
 1465 approximate the date ranges from April to June 15th (Spring management period), June 16th to
 1466 July 31st (Summer management period), and August 1st to December 31 (Fall management
 1467 period) which are used to categorize spring-, summer-, and fall-run Chinook salmon,
 1468 respectively. The number of sampled fish that were assigned via PBT or GSI are shown.

1469 Table 2: Sample numbers by monthly strata for steelhead that were DNA sampled or tallied for
 1470 abundance at Bonneville Dam in 2016.

Management Period	Statistical month (4 week strata)	Bonneville dam fish window count			Sample (N)				Sample	
					Clipped		Non-clipped			
		Total	Clipped	Non-clipped	GSI	PBT	GSI	PBT	Total	rate
Skamania Summer	15_18	818	499	319	1	6	0	0	7	0.6%
	19_22	1346	1018	328	6	32	12	0	50	3.0%
	23_26	6917	4199	2718	3	26	14	0	43	0.4%
Summer A/B Index	27_30	32340	19058	13282	13	142	125	12	292	0.6%
	31_34	53011	37352	15659	23	331	180	21	555	0.8%
	35_38	54409	44002	10407	6	207	41	22	276	0.4%
	39_42	33109	25869	7240	12	186	21	43	262	0.6%
	Total	181950	131997	49953	64	930	393	98	1485	0.6%

1471 Note: Statistical week 15 is 4/3/16–4/9/16 and 42 is 10/9/16–10/15/16 ‘Fish count’ is based on
 1472 tallies of adipose-clipped and non-clipped adult steelhead provided by the Fish Passage Center
 1473 (<http://www.fpc.org>) observed by the Corps of Engineers at their fish counting window. The
 1474 total sum of all samples for a given week was used to calculate sample rate. The management
 1475 periods approximate the date ranges from April 1st-June 30th and July 1st-October 31st which are
 1476 used to categorize Skamania and summer steelhead, respectively. The sample numbers were
 1477 split into two categories according to whether samples had been taken from fish that were
 1478 adipose clipped or non-clipped, and then further split according to the number of samples that
 1479 were either assigned via PBT or GSI.

Table 3: Sample numbers for genetic stock assignments of sockeye salmon that passed Bonneville Dam in 2016.

Statistical week grouping	Bonneville dam fish window count	OKA	WEN	RED	LBC	WAL	Total	Sample rate (%)
22_23	2462	59	9	0	0	0	68	2.76
24	15607	156	56	0	0	0	212	1.36
25	72340	264	112	0	0	1	377	0.52
26	139973	258	99	0	0	1	358	0.26
27	70448	181	78	3	0	0	262	0.37
28	24838	124	46	1	3	1	175	0.70
29	9978	82	50	2	4	1	139	1.39
30	3628	18	26	0	4	0	48	1.32
31	2098	20	15	0	1	0	36	1.72
32_33	989	5	4	1	1	0	11	1.11
Total	342361	1167	495	7	13	4	1686	0.49

Note: Statistical week 22 is 5/22/16–5/28/16 and 32 is 7/31/16–8/6/16. ‘Fish count’ is based on tallies of sockeye salmon adults provided by the Fish Passage Center (<http://www.fpc.org>) observed by the Corps of Engineers at their fish counting window. GSI stocks are Okanagan (OKA), Wenatchee (WEN), Snake River (RED), Lake Billy Chinook (LBC), and Wallowa (WAL). The number of samples for a given statistical week or grouping was used to calculate sample rate. Relatively few sockeye salmon were sampled from the RED, LBC and WAL stocks, and limits inference regarding run-timing and abundance of these stocks.

Results

Estimated relative abundance of Chinook salmon stocks in 2016

There were 11 major (i.e., abundance >1000 fish) hatchery origin Chinook salmon stocks represented in the total estimate relative abundance (N=375,646) of hatchery Chinook salmon passing Bonneville Dam in 2016 (Table 4; Figure 1). These stocks in order of decreasing magnitude were 18_UCOLSF (140,316), 19_SRFALL (69,874), 12_HELLSC (62,759), 05_SPCRTU (31,785), 10_UCOLSP (25,898), 07_DESCP (17,124), 13_SFSALM (9,558), 16_UPSALM (8,221), 09_YAKIMA (4,661), 06_KLICKR (1,669), and 15_MFSALM (1,230) (Table 4; Figure 1). Two stocks (17_DESCFA and 11_TUCANO) that were considered major in 2015 did not meet the abundance threshold (>1000) to be considered major stocks in 2016 (Table 4). The 10_UPCOLSP reporting group included Carson Hatchery for these estimates due to genetic similarity in GSI assignments, so the abundance estimate for this group does not represent actual returns in 2016 specifically to the upper Columbia River. Abundance estimates include relative abundance for PBT-assigned fish (adipose clipped and non-clipped) and adipose clipped fish that were assigned via GSI. PBT assignments improved our ability to accurately identify hatchery origin fish and estimate total stock abundance (Table 4). Further, using PBT assignments we can now provide relative abundance and run-timing estimates for particular hatchery broodstocks (Table 5; Figures 2, 5) which will allow for much improved abundance estimates including the problem noted above for the 10_UPCOLSP reporting group.

1508 Table 4: Stock-specific relative abundance and run-timing distribution of hatchery origin (adipose clipped and non-clipped) Chinook salmon passing Bonneville Dam in 2016.

Reporting Group	Estimated abundance					Run-timing distribution						
	Mean	95% CI	Management Period			Ordinal day						Interquartile range (days)
			Spring	Summer	Fall	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	
			Jan. 1-Jun. 15	Jun. 16-Jul. 31	Aug. 1-Dec. 1							
01_YOUNGS¶	400	32 – 1600	0	0	400	-	-	-	-	-	-	-
02_WCASSP¶	0	3 – 758	0	0	0	-	-	-	-	-	-	-
03_WCASFA¶	147	126 – 1470	0	0	147	-	-	-	-	-	-	-
04_WILLAM¶	326	85 – 1580	326	0	0	-	-	-	-	-	-	-
05_SPCRTU	31785	25267 – 36748	0	0	31785	246	239	250	233	258	09/03/16	11
06_KLICKR	1669*	963 – 2595	1316	353	0	145	137	160	118	167	05/25/16	23
07_DESCSP	17124*	14096 – 20369	16975	0	149	124	121	134	113	152	05/04/16	13
08_JOHNDR¶	348	26 – 1357	348	0	0	-	-	-	-	-	-	-
09_YAKIMA	4661*	3504 – 6732	4337	324	0	133	122	145	103	171	05/13/16	23
10_UCOLSP@	25898*	23515 – 30403	25693	205	0	121	118	127	104	142	05/01/16	9
11_TUCANO¶	652*	154 – 1576	652	0	0	-	-	-	-	-	-	-
12_HELLSC	62759*	59237 – 67022	62438	321	0	122	119	127	111	147	05/02/16	8
13_SFSALM	9558*	8170 – 12408	8403	1155	0	150	138	160	127	172	05/30/16	22
14_CHMBLN¶	0	0 – 554	0	0	0	-	-	-	-	-	-	-
15_MFSALM¶	1230	456 – 2864	906	324	0	-	-	-	-	-	-	-
16_UPSALM	8221*	6505 – 10913	7282	826	112	143	131	154	121	187	05/23/16	23
17_DESCFA¶	678	1 – 2491	0	73	605	-	-	-	-	-	-	-
18_UCOLSF	140316*	127321 – 147186	12267	35276	92773	239	187	253	161	263	08/27/16	66
19_SRFALL	69874*	60380 – 76757	0	1417	68457	243	236	250	219	264	08/31/16	14
Total	375646*		140943	40274	194428							

1509 *Combined GSI and PBT estimated abundance

1510 [@]The 10_UPCOLSP reporting group included Carson Hatchery for these estimates due to genetic similarity in GSI assignments, so the abundance estimate for this group does not
1511 represent actual returns in 2016 specifically to the upper Columbia River.

1512 [¶]Run-timing distributions were not estimated for stocks where the combined number of PBT and GSI assignments to reporting group was n<5.

1513

1514 **Note:** These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on weekly stock proportions and total numbers of
1515 Chinook salmon that were observed passing Bonneville Dam at the fish counting window. The run-timing distributions are characterized by ordinal days for the median date, inter-quartile
1516 range (days), and 5th and 95th percentile. The distributions were based on the weekly estimated reporting group proportions that were applied to the daily tallies of Chinook salmon at the
1517 Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Run-timing distributions for hatchery-origin
1518 fish include stock abundance estimated from PBT and GSI assignments.

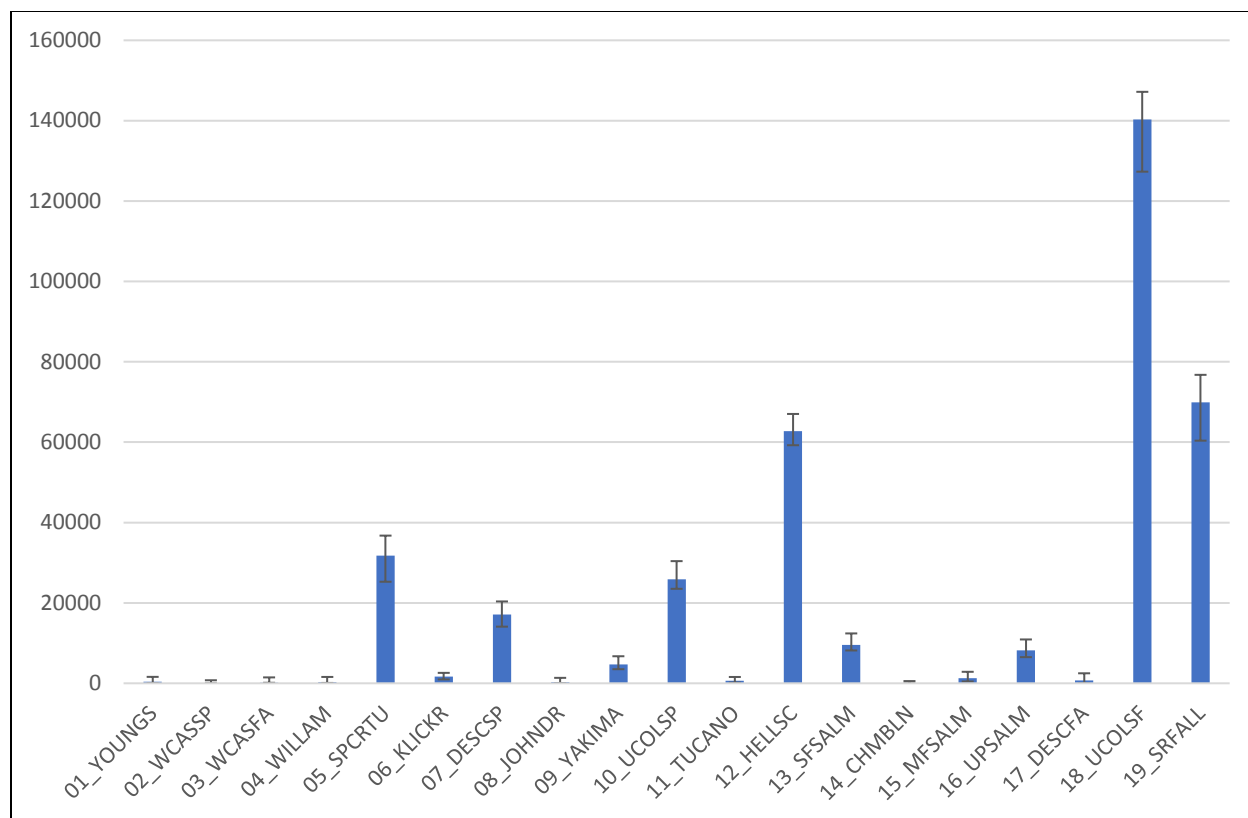


Figure 1: Relative abundance (\pm 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2016. **Note:** The 10_UPCOLSP reporting group included Carson Hatchery for these estimates due to genetic similarity in GSI assignments, so the abundance estimate for this group does not represent actual returns in 2016 specifically to the upper Columbia River.

We detected PBT assignments for 15% (254/1692) of adipose non-clipped (i.e., presumed natural-origin) Chinook salmon sampled at Bonneville Dam in 2016. There were 12 major (i.e., abundance >1000 fish) Chinook salmon stocks represented in the total estimated relative abundance (N=390,320) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) Chinook salmon passing Bonneville Dam in 2016 (Table 6; Figure 3). These non-clipped stocks in order of decreasing magnitude were 18_UCOLSF (278,984), 19_SRFALL (63,642), 12_HELLSC (9,118), 05_SPCRTU (6,773), 17_DESCFA (6,497), 09_YAKIMA (4,441), 16_UPSALM (4,426), 15_MFSALM (3,416), 13_SFSALM (2,915), 10_UCOLSP (2,836), 08_JOHNDR (2,378), and 07_DESCP (1,769). The 10_UPCOLSP reporting group included Carson Hatchery for these estimates due to genetic similarity in GSI assignments, so the abundance estimate for this group does not represent actual returns in 2016 specifically to the upper Columbia River. These stock abundance estimates were based on the stock proportions that were estimated in GSI_sim across weekly strata, and were multiplied by the total abundance of Chinook salmon that was tallied on a daily basis at the Bonneville Dam fish counting window (Table 1).

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Table 5: Hatchery broodstock-specific relative abundance and run-timing distributions of adipose clipped and non-clipped PBT-assigned Chinook salmon passing Bonneville Dam in 2016. Key to broodstock collection is presented in Section 3 (Table 1).

Reporting Group	Broodstock collection	Tagging rate (%)	Estimated abundance					Run-timing distribution						
			Mean	95% CI	Management Period			Ordinal day						
					Spring	Summer	Fall	Median	1st	3rd	5th	95th	Median	Interquartile
					Jan. 1-Jun. 15	Jun. 16-Jul. 31	Aug. 1-Dec. 1		quartile	quartile	percentile	percentile	date	range (days)
06_Klickr	OtsKH11S*	80.72	240	0 – 625	76	163	0	-	-	-	-	-	-	-
	OtsKH13S	97.88	540	180 – 1034	540	0	0	138	134	147	127	160	05/18/16	13
07_DESCSP [†]	OtsPFF12	89.00	1536	640 – 2657	1536	0	0	123	121	130	118	158	05/03/16	9
	OtsRB12	92.00	2062	984 – 3329	2062	0	0	124	121	134	118	141	05/04/16	13
	OtsWSNFH12	60.00	8031	5710 – 10608	8031	0	0	126	121	136	111	155	05/06/16	15
	OtsRB13	94.00	481	98 – 1049	481	0	0	142	122	148	120	153	05/22/16	26
	OtsWSNFH13*	75.66	257	0 – 649	257	0	0	-	-	-	-	-	-	-
	OtsPFF13*	68.00	154	0 – 485	154	0	0	-	-	-	-	-	-	-
	OtsPFF14*	100.00	149	0 – 447	0	0	149	-	-	-	-	-	-	-
09_YAKIMA	OtsYR12	99.37	2403	1306 – 3618	2403	0	0	122	118	134	101	142	05/02/16	16
10_UCOLSP	OtsCAR12 [®]	90.00	9020	6758 – 11602	9020	0	0	121	114	124	103	132	05/01/16	10
	OtsMETH12	93.00	1076	334 – 2032	870	205	0	127	121	164	117	167	05/07/16	43
	OtsUMA12_sp [®]	92.00	1577	641 – 2665	1577	0	0	120	117	124	110	131	04/30/16	7
	OtsLWS13_sp* [®]	90.49	385	53 – 869	385	0	0	-	-	-	-	-	-	-
	OtsWTP13	85.17	861	392 – 1396	861	0	0	137	132	142	127	146	05/17/16	10
	OtsLNFH13*	88.03	133	0 – 428	133	0	0	-	-	-	-	-	-	-
	OtsMETH13*	94.00	119	0 – 303	119	0	0	-	-	-	-	-	-	-
	OtsUMA13_sp [®]	96.00	863	257 – 1655	863	0	0	127	122	133	120	138	05/07/16	11
11_TUCANO	OtsLYON12S	95.23	652	101 – 1358	652	0	0	124	121	145	120	160	05/04/16	24
12_HELLSC*	OtsCLWH11S*	96.15	287	0 – 742	287	0	0	-	-	-	-	-	-	-
	OtsCLWH12S	92.02	3441	2025 – 5056	3441	0	0	123	121	128	118	135	05/03/16	7
	OtsCTHW11S*	92.16	133	0 – 406	133	0	0	-	-	-	-	-	-	-
	OtsDWOR11S	98.99	779	127 – 1563	779	0	0	123	121	126	120	142	05/03/16	5
	OtsDWOR12S	99.26	18866	16047 – 22019	18866	0	0	121	119	125	113	137	05/01/16	6
	OtsLOOK11S*	97.47	179	0 – 554	179	0	0	-	-	-	-	-	-	-
	OtsLOOK12S	98.35	6888	5049 – 8710	6567	321	0	131	122	152	120	163	05/11/16	30
	OtsLSTW11S*	91.53	216	0 – 650	216	0	0	-	-	-	-	-	-	-
	OtsPOWP11S*	98.69	516	0 – 2657	516	0	0	-	-	-	-	-	-	-
	OtsPOWP12S	98.66	5403	3779 – 7193	5403	0	0	120	115	123	104	134	04/30/16	8
	OtsRAPH11S	98.35	2474	1366 – 3765	2474	0	0	122	121	126	111	133	05/02/16	5
	OtsRAPH12S	95.36	17348	14572 – 20483	17348	0	0	120	118	124	110	137	04/30/16	6
	OtsGRUW13S*	96.07	203	0 – 617	203	0	0	-	-	-	-	-	-	-
	OtsCLWH13S	93.96	892	312 – 1547	892	0	0	131	127	137	121	145	05/11/16	10
	OtsLSTW13S*	95.18	113	0 – 354	113	0	0	-	-	-	-	-	-	-
	OtsIMNW13S*	97.89	131	0 – 391	131	0	0	-	-	-	-	-	-	-
13_SFSALM	OtsJHNW12S*	93.40	262	0 – 659	262	0	0	-	-	-	-	-	-	-
	OtsMCCA11S	99.04	1200	513 – 2033	1008	192	0	145	137	164	130	167	05/25/16	27
	OtsMCCA12S	97.10	7086	5416 – 8977	6123	963	0	148	137	160	125	173	05/28/16	23
16_UPSALM	OtsPAHH11S*	96.62	100	0 – 253	100	0	0	-	-	-	-	-	-	-
	OtsSAWT11S	96.97	646	123 – 1274	646	0	0	130	124	136	120	153	05/10/16	12
	OtsSAWT12S	93.69	5183	3728 – 6848	4838	345	0	139	130	152	121	185	05/19/16	22
	OtsSAWT13S*	99.17	124	0 – 372	124	0	0	-	-	-	-	-	-	-
	OtsPAHH12S	99.51	686	210 – 1303	373	201	112	160	150	180	142	216	06/09/16	30
18_UCOLSF	OtsPRH12	62.64	20423	13739 – 27055	0	0	20423	248	242	254	229	268	09/05/16	12
	OtsWELLS12	99.47	5029	3322 – 6855	1762	2831	436	167	164	179	156	230	06/16/16	15
	OtsEASTBK12	96.19	4359	2961 – 5877	702	3466	191	185	174	192	154	210	07/04/16	18
	OtsWELLS13	97.69	1614	677 – 2723	126	1488	0	181	175	189	159	197	06/30/16	14
	OtsCJH14_sufa*	99.14	89	0 – 269	0	89	0	-	-	-	-	-	-	-
	OtsPRH13	79.12	11389	7149 – 16294	0	0	11389	253	245	258	239	272	09/10/16	13
	OtsPRO12**	89.50	1085	0 – 2822	0	0	1085	-	-	-	-	-	-	-
	OtsLWS14_sufa**	92.92	616	0 – 1664	0	0	616	-	-	-	-	-	-	-
	OtsPRH14*	86.77	2293	192 – 5193	0	0	2293	-	-	-	-	-	-	-
	OtsUMA12_sufa**	99.00	733	0 – 1791	0	0	733	-	-	-	-	-	-	-
19_SRFALL	OtsLYON11S	89.88	2635	1028 – 4581	0	0	2635	231	224	242	213	275	08/19/16	18
	OtsLYON12S_1	98.60	15320	10904 – 20531	0	0	15320	241	236	245	227	257	08/29/16	9
	OtsNPFH11S_1	89.88	3351	1188 – 6032	0	101	3250	248	228	250	213	263	09/05/16	22
	OtsNPFH12S_1	99.37	8660	5081 – 13238	0	177	8483	246	230	249	221	256	09/03/16	19
	OtsLYON14S_1	97.75	8675	5545 – 12317	0	0	8675	242	231	253	225	265	08/30/16	22
	OtsNPFH13S_1	99.27	8877	5258 – 12974	0	0	8877	244	238	250	226	278	09/01/16	12
	OtsLYON13S_1	97.04	14494	9763 – 19800	0	0	14494	248	242	256	233	265	09/05/16	14
	OtsNPFH14S_1*	99.15	867	0 – 2081	0	0	867	-	-	-	-	-	-	-
	Total		214204		103635	10542	100029							

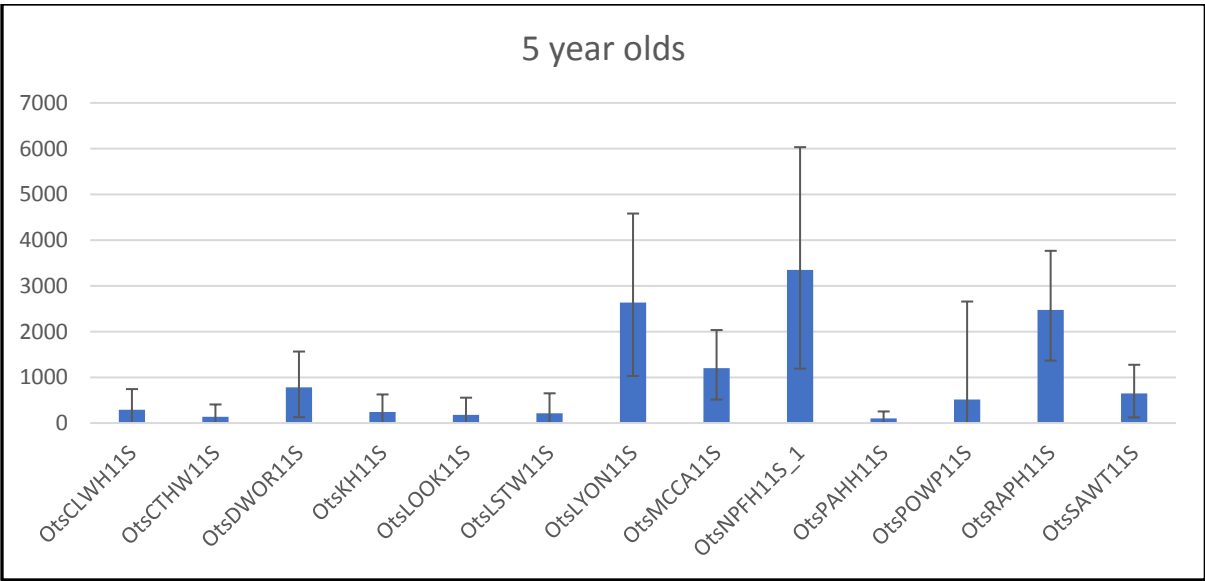
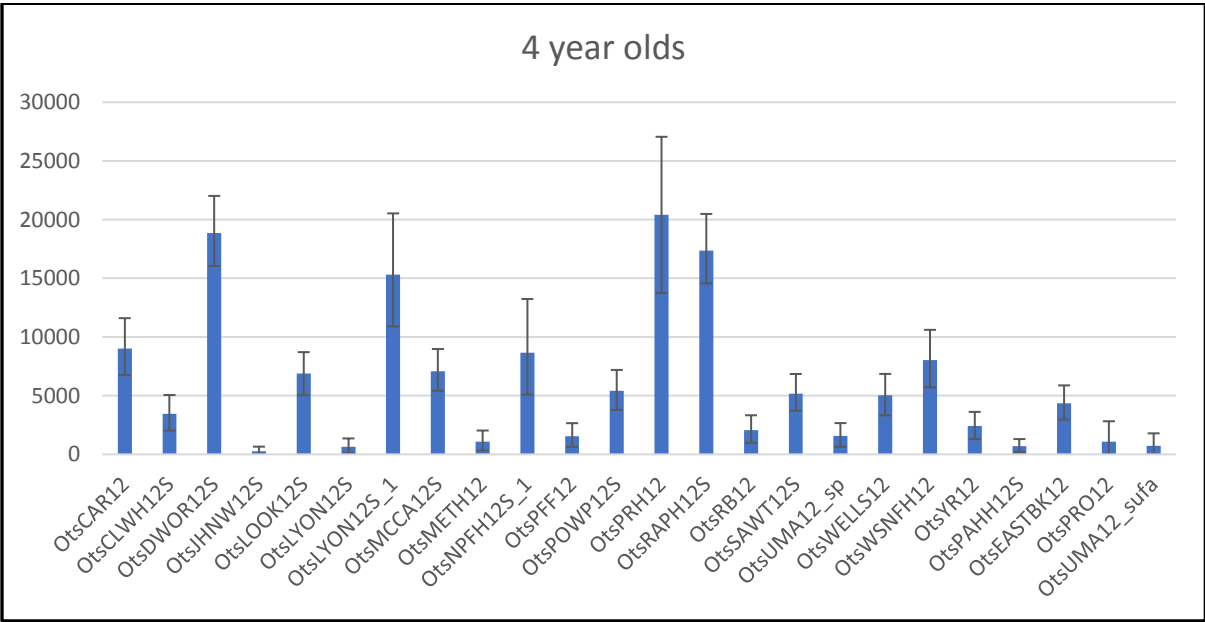
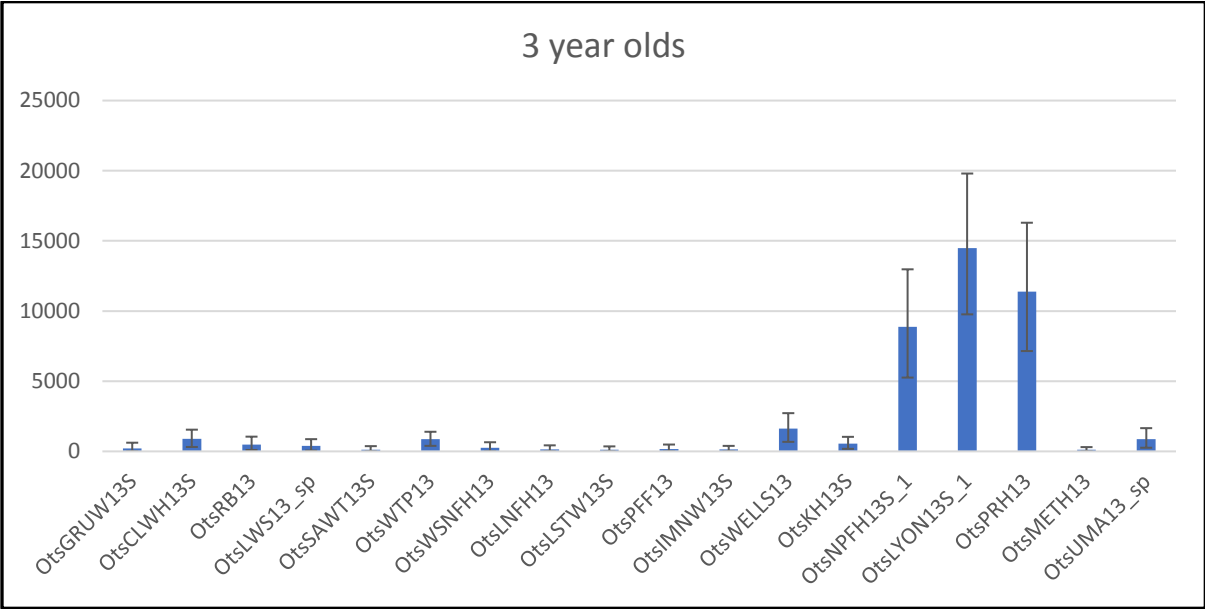
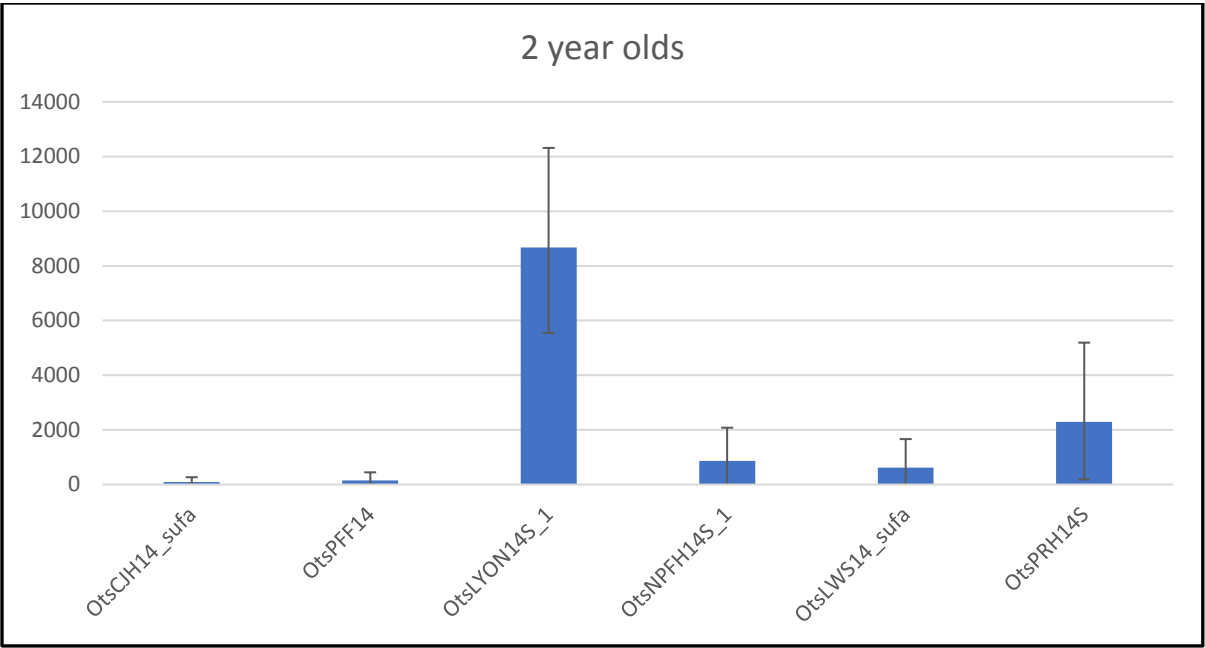


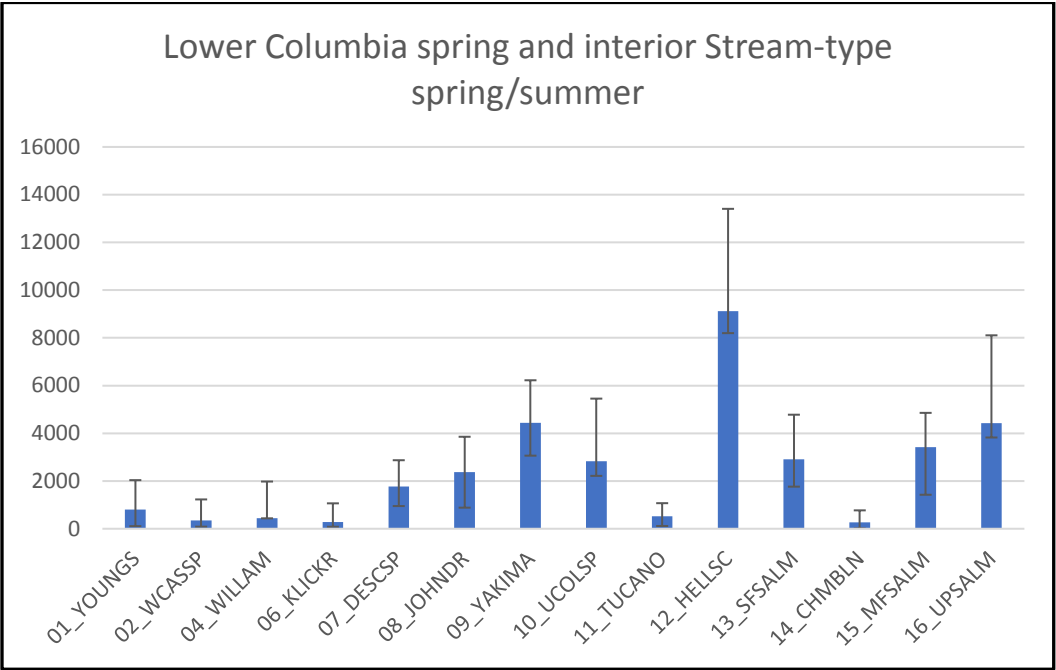
Figure 2: Relative abundance (\pm 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) sampled at Bonneville Dam in 2016 that assigned via PBT to 61 hatchery broodstocks of origin by age class. The 2014 age-class (2-year old fish; top panel), 2013 age class (3-year old fish), 2012 age class (4-year old fish), and 2011 age class (5-year old fish) are shown. Key to broodstock collection is presented in Section 3 (Table 1)

1559 Table 6: Relative abundance and run-timing distributions of natural origin (adipose non-clipped) Chinook salmon stocks passing Bonneville Dam in 2016.

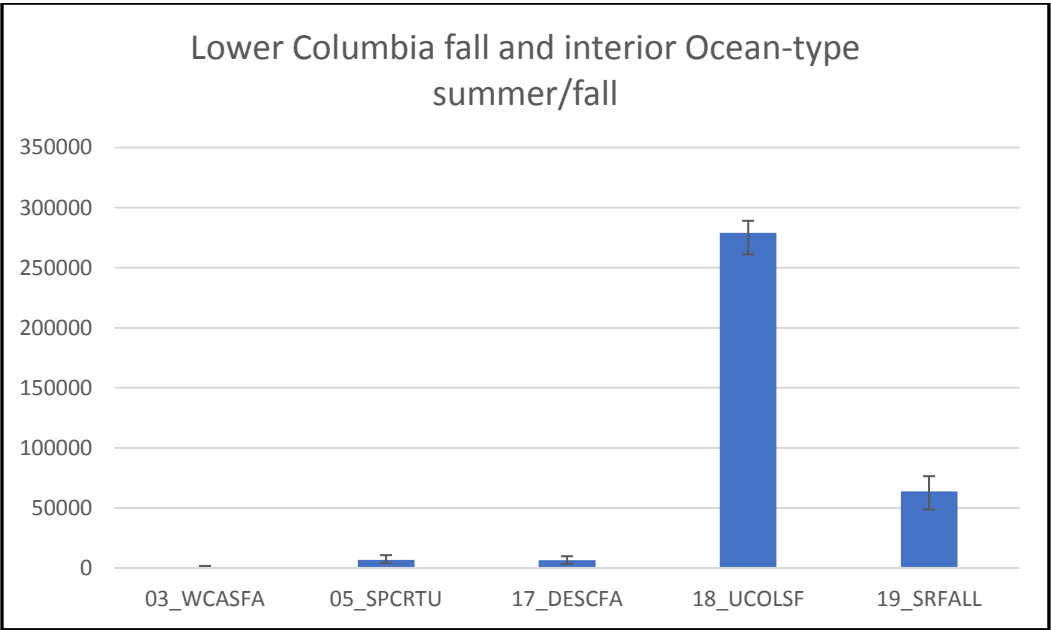
Reporting Group	Estimated abundance					Run-timing distribution						
	Mean	95% CI	Management Period			Ordinal day						
			Spring	Summer	Fall	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (days)
			Jan. 1-Jun. 15	Jun. 16-Jul. 31	Aug. 1-Dec. 1							
01_YOUNGS*	803	110 – 2037	0	0	803	-	-	-	-	-	-	-
02_WCASSP*	351	88 – 1229	162	189	0	-	-	-	-	-	-	-
03_WCASFA*	456	108 – 1696	0	0	456	-	-	-	-	-	-	-
04_WILLAM*	437	165 – 1980	0	0	437	-	-	-	-	-	-	-
05_SPCRTU	6773	4157 – 10642	0	87	6686	245	242	255	229	264	09/02/16	13
06_KLICKR*	286	85 – 1065	286	0	0	-	-	-	-	-	-	-
07_DESCSP	1769	951 – 2872	1769	0	0	127	118	135	110	157	05/07/16	17
08_JOHNDR*	2378	884 – 3855	2189	189	0	-	-	-	-	-	-	-
09_YAKIMA	4441	3062 – 6222	4441	0	0	127	121	136	118	158	05/07/16	15
10_UCOLSP	2836	2216 – 5454	2836	0	0	126	121	139	110	154	05/06/16	18
11_TUCANO*	528	116 – 1072	528	0	0	-	-	-	-	-	-	-
12_HELLSC	9118	8197 – 13407	8792	326	0	130	120	144	114	161	05/10/16	24
13_SFSALM	2915	1765 – 4779	2514	401	0	143	136	158	128	184	05/23/16	22
14_CHMBLN*	264	16 – 772	264	0	0	-	-	-	-	-	-	-
15_MFSALM*	3416	1425 – 4856	3006	410	0	-	-	-	-	-	-	-
16_UPSALM	4426	3825 – 8107	4233	194	0	135	123	150	120	161	05/15/16	27
17_DESCFA	6497	3415 – 9733	0	329	6168	259	244	266	203	274	09/16/16	22
18_UCOLSF	278984	261123 – 289064	13026	47247	218711	248	230	257	166	270	09/05/16	27
19_SRFALL	63642	48688 – 76396	176	1758	61707	247	239	252	219	266	09/04/16	13
Total	390320		44220	51130	294968							

1560 * Run-timing distributions were not estimated for stocks where the number of GSI assignments was n<5.

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Figure 3: Relative abundance (\pm 95% CI) of natural origin (adipose non-clipped) Chinook sampled at Bonneville Dam in 2016 assigned to genetic stock of origin. Lower Columbia spring and interior stream-type spring/summer Chinook reporting groups (top panel), and lower Columbia fall and interior Ocean-type Chinook reporting groups (bottom panel) are shown.

Run-timing of Chinook salmon stocks in 2016

We obtained a sufficient number of PBT and GSI assignments to characterize the run-timing distributions of 10 hatchery-origin (adipose clipped and non-clipped) Chinook salmon stocks (Table 4; Figure 4). The run timing of three hatchery-origin spring Chinook stocks (i.e., 07_DESCSP, 10_UCOLSP, and 12_HELLSC) were found to terminate within the spring management period (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run timing for three hatchery-origin spring Chinook stocks (i.e., 06_KLICKR, 09_YAKIMA, and 13_SFSALM), and one hatchery spring/summer Chinook stock (i.e., 16_UPSALM) were found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The run-timing for the remaining hatchery summer/fall Chinook (i.e., 18_UCOLSF) and hatchery fall Chinook stocks (i.e., 05_SPCRTU and 19_SRFALL) all had median dates on or after 8/27/16 (Table 4).

We obtained a sufficient number of GSI assignments to characterize the run-timing distributions of 10 natural-origin (adipose non-clipped) Chinook salmon stocks (Table 6; Figure 4). The run timing of four natural-origin spring Chinook stocks (i.e., 07_DESCP, 09_YAKIMA, 10_UCOLSP, and 12_HELLSC) and one spring/summer Chinook stock (i.e., 16_UPSALM) were found to terminate within the spring management period (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run timing for one natural-origin spring Chinook stocks (i.e., 13_SFSALM) was found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The run-timing for one natural-origin summer/fall Chinook stock (i.e., 18_UCOLSF) and three natural-origin fall Chinook salmon stocks (i.e., 05_SPCRTU, 17_DESCFA, and 19_SRFALL) all had median dates on or after 9/2/16 (Table 6).

Using the PBT-assigned Chinook salmon, we also characterized the run timing distributions for hatchery broodstocks in our PBT baseline (Table 5; Figure 5). Among major broodstock sources (mean estimated abundance ≥ 1000 fish), the run-timing of 12 broodstocks (i.e., OtsUMA12_sp, OtsCAR12, OtsRAPH11S, OtsRAPH12S, OtsPOWP12S, OtsCLWH12S, OtsDWOR12S, OtsRB12, OtsYR12, OtsWSNFH12, OtsPFF12, and OtsLOOK12S) were found to terminate within the spring management (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run-timing for six broodstocks (i.e., OtsMCCA11S, OtsMCCA12S, OtsSAWT12S, OtsMETH12, OtsWELLS13, and OtsEASTBK12) were found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The 95th percentile of the run timing distribution for the OtsWELLS12 broodstock occurred on ordinal day 230 (08/08/16), but a median run timing date of 06/16/16. The run-timing for the nine remaining broodstock sources (i.e., OtsLYONS11S, OtsLYON12S_1, OtsLYON13S_1, OtsLYON14S_1, OtsNPFH11S_1, OtsNPFH12S_1, OtsNPFH13S_1, OtsPRH12, OtsPRH13, OtsPRH14, and OtsPRO12) all had median dates on or after 09/03/16 (Table 5).

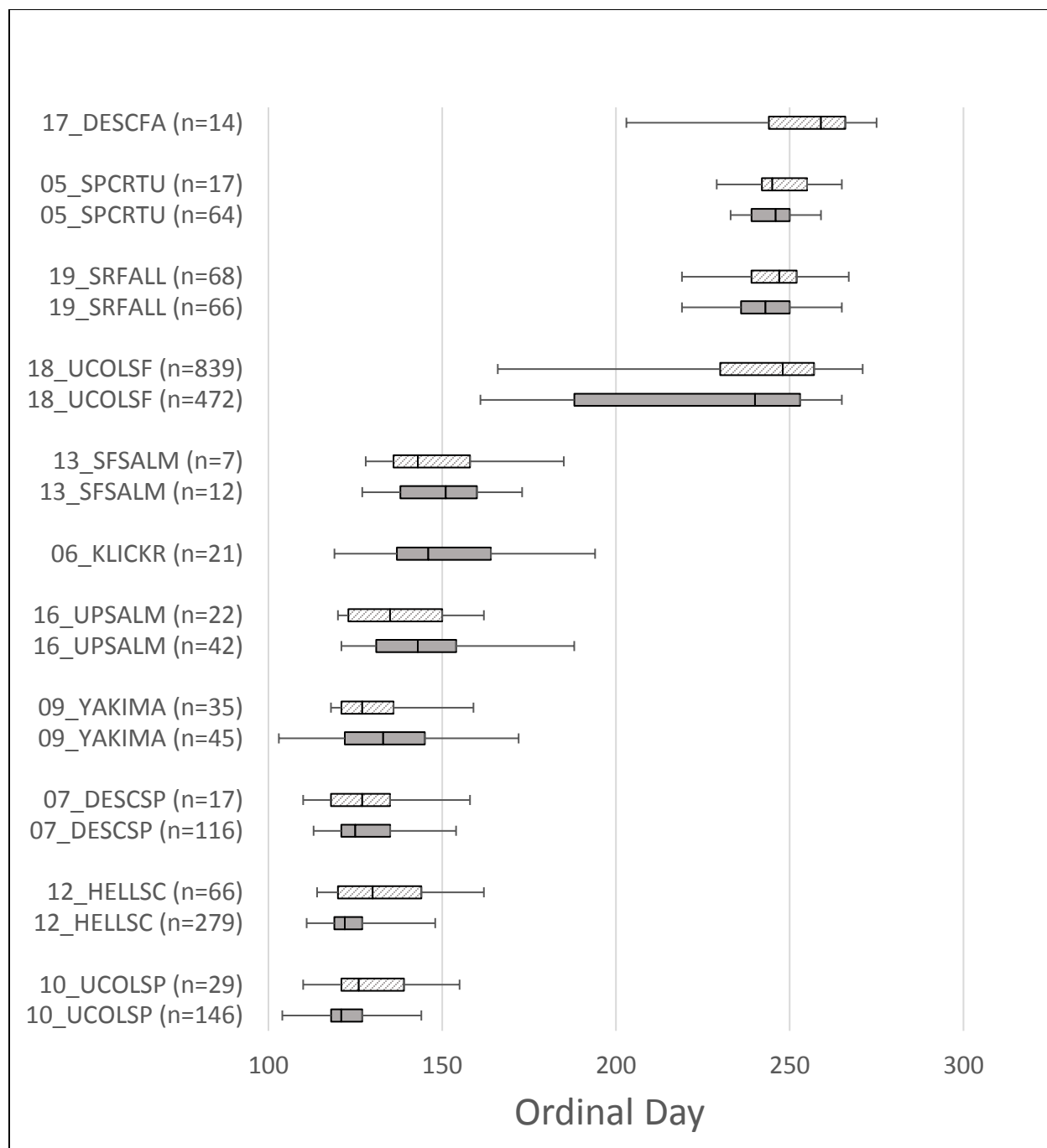


Figure 4: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin Chinook (adipose clipped and non-clipped; solid filled) and natural origin Chinook (adipose non-clipped; hash-marked) that were sampled at Bonneville Dam in 2016 and assigned to stock of origin. The number of PBT and/or GSI assignments used in the relative stock abundance expansion procedure from which these run-timing distributions are derived are shown.

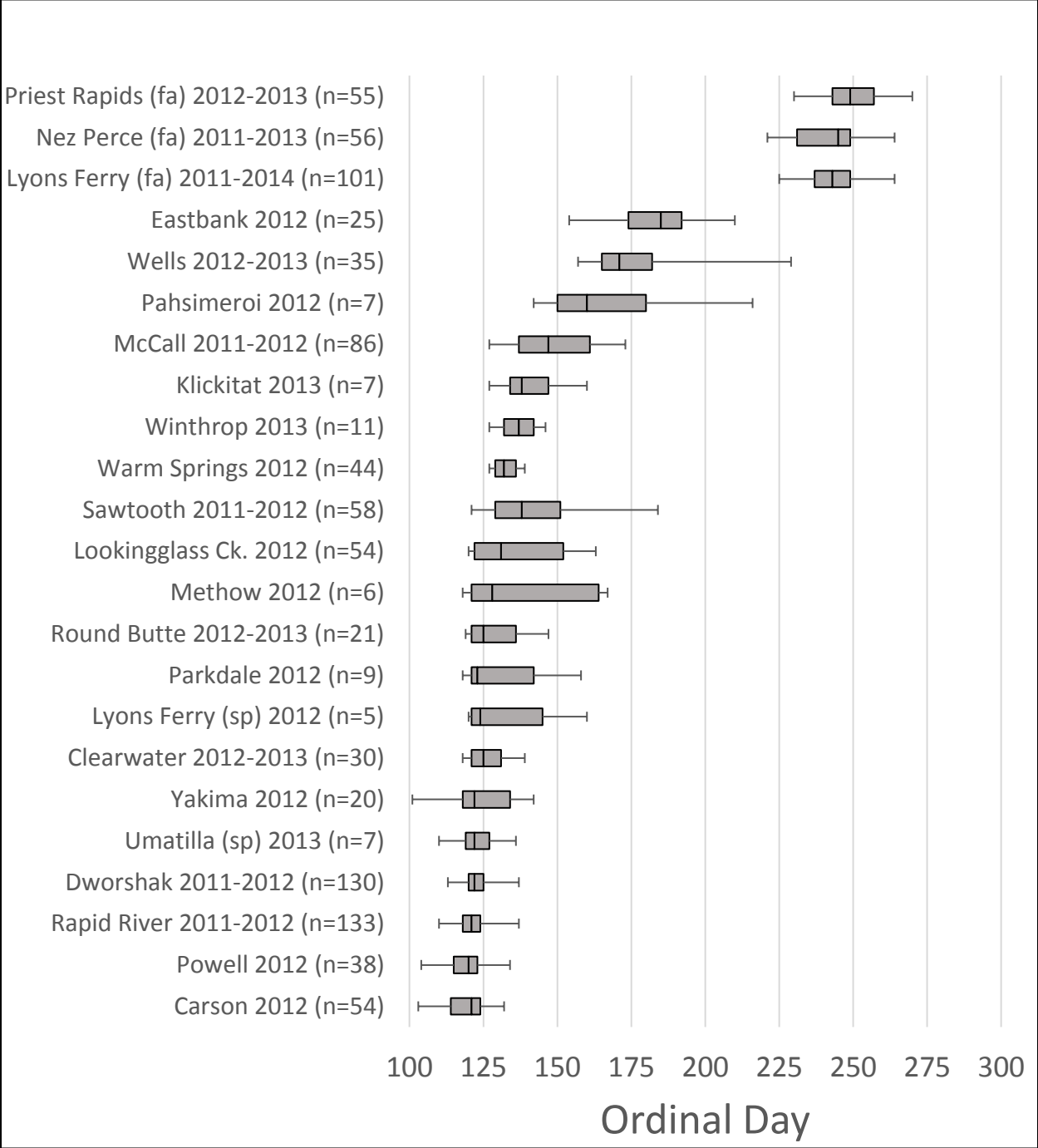


Figure 5: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin Chinook (adipose clipped and non-clipped) assigned to PBT broodstock of origin that were sampled at Bonneville Dam in 2016. Hatcheries and their broodyears are ordered from earliest to latest median run-timing, with fall Chinook hatchery stocks at the top. The number of PBT assignments used in the relative stock abundance expansion procedure from which these run-timing distributions are derived are shown.

PBT and GSI assignments for Chinook salmon in 2016

We were able to assign 1046 adult and jack Chinook salmon sampled at Bonneville Dam in 2016 to 61 different hatchery broodstock sources from 2011-2014. The majority (i.e., 716; 68.5%) assigned to 33 Snake River hatchery broodstock sources, while the remaining 330 fish assigned to 28 Columbia River hatchery broodstock sources (Table 7). The Snake River and Columbia River hatchery broodstock sources were aggregated into appropriate GSI reporting groups in order to integrate the relative abundance estimates from this analysis with relative abundance from GSI analyses. Tagging rates varied across hatchery brood stock sources from 60% to 100%, with 34 hatchery broodstock sources having tagging rates <95% (Table 7).

Using GSI, we were able to assign 1129 natural-origin and 670 hatchery-origin adult and jack Chinook salmon (that could not be assigned via PBT) sampled at Bonneville Dam in 2016 to 19 reporting groups throughout the Columbia River basin (Table 8). The majority (72%) of these assignments were to the interior Ocean-type lineage (dominated by assignments to 18_UCOLSF; 93%), followed by assignment to the interior Stream-type lineage (23%), and lower Columbia lineage (5%) (Table 8).

1 Table 7: Summary information about the PBT Chinook salmon hatchery broodstock sources and number
2 of assignments. Key to broodstock collection is presented in Section 3 (Table 1).

Broodstock collection	Basin	Reporting Group	Tagging rate (%)	Age	Adipose fin		Total
					Clipped	Non-clipped	
OtsKH11S	Columbia	06_KLICKR	80.72	5	2		2
OtsKH13S	Columbia	06_KLICKR	97.88	3	7		7
OtsPFF12	Columbia	07_DESCSP	89.00	4	10		10
OtsPFF13	Columbia	07_DESCSP	68.00	3	1		1
OtsPFF14	Columbia	07_DESCSP	100.00	2	1		1
OtsRB12	Columbia	07_DESCSP	92.00	4	16		16
OtsRB13	Columbia	07_DESCSP	94.00	3	5		5
OtsWSNFH12	Columbia	07_DESCSP	60.00	4	42	2	44
OtsWSNFH13	Columbia	07_DESCSP	75.66	3	2		2
OtsYR12	Columbia	09_YAKIMA	99.37	4	18	2	20
OtsCAR12 [@]	Columbia	10_UCOLSP	90.00	4	53	1	54
OtsLNFH13	Columbia	10_UCOLSP	88.03	3	1		1
OtsLWS13_sp [@]	Columbia	10_UCOLSP	90.49	3	4		4
OtsMETH12	Columbia	10_UCOLSP	93.00	4		6	6
OtsMETH13	Columbia	10_UCOLSP	94.00	3		2	2
OtsUMA12_sp [@]	Columbia	10_UCOLSP	92.00	4	7	3	10
OtsUMA13_sp [@]	Columbia	10_UCOLSP	96.00	3	6	1	7
OtsWTP13	Columbia	10_UCOLSP	85.17	3	9	2	11
OtsLYON12S	Snake	11_TUCANO	95.23	4		5	5
OtsCLWH11S	Snake	12_HELLSC	96.15	5	2		2
OtsCLWH12S	Snake	12_HELLSC	92.02	4	19	2	21
OtsCLWH13S	Snake	12_HELLSC	93.96	3	7	2	9
OtsCTHW11S	Snake	12_HELLSC	92.16	5	1		1
OtsDWOR11S	Snake	12_HELLSC	98.99	5	4	1	5
OtsDWOR12S	Snake	12_HELLSC	99.26	4	115	10	125
OtsGRUW13S	Snake	12_HELLSC	96.07	3	1		1
OtsIMNW13S	Snake	12_HELLSC	97.89	3	1		1
OtsLOOK11S	Snake	12_HELLSC	97.47	5	1		1
OtsLOOK12S	Snake	12_HELLSC	98.35	4	46	8	54
OtsLSTW11S	Snake	12_HELLSC	91.53	5	1		1
OtsLSTW13S	Snake	12_HELLSC	95.18	3	1		1
OtsPOWP11S	Snake	12_HELLSC	98.69	5	2	1	3
OtsPOWP12S	Snake	12_HELLSC	98.66	4	31	6	37
OtsRAPH11S	Snake	12_HELLSC	98.35	5	16		16
OtsRAPH12S	Snake	12_HELLSC	95.36	4	114	3	117
OtsJHNW12S	Snake	13_SFSALM	93.40	4		2	2
OtsMCCA11S	Snake	13_SFSALM	99.04	5	8	5	13
OtsMCCA12S	Snake	13_SFSALM	97.10	4	40	33	73
OtsPAHH11S	Snake	16_UPSALM	96.62	5	2		2
OtsPAHH12S	Snake	16_UPSALM	99.51	4	6	1	7
OtsSAWT11S	Snake	16_UPSALM	96.97	5	4	1	5
OtsSAWT12S	Snake	16_UPSALM	93.69	4	43	10	53
OtsSAWT13S	Snake	16_UPSALM	99.17	3	1		1
OtsCJH14_sufa	Columbia	18_UCOLSF	99.14	2	1		1
OtsEASTBK12	Columbia	18_UCOLSF	96.19	4	24	1	25
OtsLWS14_sufa [*]	Columbia	18_UCOLSF	92.92	2		2	2
OtsPRH12	Columbia	18_UCOLSF	62.64	4	14	17	31
OtsPRH13	Columbia	18_UCOLSF	79.12	3	9	15	24
OtsPRH14S	Columbia	18_UCOLSF	86.77	2		4	4
OtsPRO12 [*]	Columbia	18_UCOLSF	89.50	4		2	2
OtsUMA12_sufa [*]	Columbia	18_UCOLSF	99.00	4	2	1	3
OtsWELLS12	Columbia	18_UCOLSF	99.47	4	21	5	26
OtsWELLS13	Columbia	18_UCOLSF	97.69	3	9		9
OtsLYON11S	Snake	19_SRFALL	89.88	5	5	5	10
OtsLYON12S_1	Snake	19_SRFALL	98.60	4	18	18	36
OtsLYON13S_1	Snake	19_SRFALL	97.04	3	12	19	31
OtsLYON14S_1	Snake	19_SRFALL	97.75	2	14	10	24
OtsNPFH11S_1	Snake	19_SRFALL	89.88	5	2	8	10
OtsNPFH12S_1	Snake	19_SRFALL	99.37	4	3	20	23
OtsNPFH13S_1	Snake	19_SRFALL	99.27	3	7	16	23
OtsNPFH14S_1	Snake	19_SRFALL	99.15	2	1	2	3

3 [@]Actual location is in Lower Columbia, but is included in the 10_UCOLSP reporting group due to
4 genetic similarities in GSI assignments.
5 ^{*}Actual location is in Lower Columbia, but is included in the 18_UCOLSF reporting group due to
6 genetic similarities in GSI assignments.

7 Table 8: Summary of the number of GSI assignments by reporting group for hatchery-origin
8 Chinook salmon that did not assign via PBT, and natural-origin Chinook salmon that assigned
9 via GSI.

Reporting group	Run type	Lineage	Adipose fin		Total
			Clipped	Non-clipped	
01_YOUNGS	Spring	Lower Columbia	1	2	3
02_WCASSP	Spring	Lower Columbia		3	3
03_WCASFA	Fall	Lower Columbia	1		1
04_WILLAM	Spring	Lower Columbia	1	2	3
05_SPCRTU	Fall	Lower Columbia	64	17	81
06_KLICKR	Spring	Interior stream type	12	3	15
07_DESCSP	Spring	Interior stream type	44	17	61
08_JOHNDR	Spring	Interior stream type			
09_YAKIMA	Spring	Interior stream type	26	35	61
10_UCOLSP	Spring	Interior stream type	89	29	118
11_TUCANO	Spring	Interior stream type		2	2
12_HELLSC	Spring	Interior stream type	52	66	118
13_SFSALM	Spring	Interior stream type		7	7
14_CHMBLN	Spring	Interior stream type			
15_MFSALM	Spring	Interior stream type	1	3	4
16_UPSALM	Spring/Summer	Interior stream type	10	22	32
17_DESCFA	Fall	Interior ocean type	1	14	15
18_UCOLSF	Summer/Fall	Interior ocean type	361	839	1200
19_SRFALL	Fall	Interior ocean type	7	68	75
		Total	670	1129	1799

10

Estimated relative abundance of steelhead stocks in 2016

There were five major stocks (abundance >1000) represented in the total estimated relative abundance (N=142,411) of hatchery origin steelhead passing Bonneville Dam in 2016 (Table 9). These stocks in order of decreasing magnitude were 10_SFCLWR (51,452), 14_UPSALM (38,629), 07_MGILCS (36,137), 03_SKAMAN (8,673), and 09_UPPCOL (7,084) (Table 9; Figure 6). These same stocks were identified as being of major abundance in 2015. These estimates include relative abundance estimated from PBT-assigned fish that were mostly adipose clipped; however, a portion of the PBT-assigned fish were found to be non-clipped. Therefore, PBT assignments improved our ability to accurately identify hatchery-origin steelhead and estimate total stock relative abundance. Further, using PBT assignments we can now provide relative abundance (Table 10; Figure 7) and run-timing estimates for particular hatchery broodstocks (Table 10). There were 15 major hatchery broodstock sources (abundance >1000) represented in the total estimated relative abundance of hatchery origin steelhead passing Bonneville Dam in 2016 (Table 10). These stocks in order of decreasing magnitude were OmyDWOR13S (49,712), OmyPAHH13S (15,956), OmyLYON13S (10,704), OmyWALL13S (8,313), OmySKH12_su (7,583), OmyOXBO13S (6,936), OmySAWT13S (6,888), OmyLYON14S (5,411), OmyWEL13 (5,230), , OmyPAHH14S (5,039), OmyRB13 (4,260), OmySAWT14S (2,414), OmyWALL14S (1,659), OmyOXBO14S (1,176), and OmyLSCR13S (1,103). Skamania broodstock are spawned earlier in the year than other broodstock collections, and are designated for the following brood year (e.g., OmySKH12_su are spawned in 2013 and are 2-ocean fish).

We detected PBT assignments for 20% (98/491) of adipose non-clipped (i.e., presumed natural-origin) steelhead sampled at Bonneville Dam in 2016. There were eight major stocks (abundance >1000) represented in the total estimated relative abundance (N=39,218) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) steelhead passing Bonneville Dam in 2016 (Table 11; Figure 8). These stocks in order of decreasing magnitude were 07_MGILCS (25,335), 06_KLICKR (2,372), 11_UPCLWR (2,300), 08_YAKIMA (2,003), 14_UPSALM (1,937), 13_MFSALM (1,443), 09_UPPCOL (1,133), and 02_LOWCOL (1,1015).

39 Table 9: Stock-specific relative abundance and run-timing distribution of hatchery origin (adipose clipped and non-clipped) steelhead passing Bonneville Dam in 2016.

Reporting Group	Estimated abundance				Run-timing distribution						
	Mean	95% CI	Management Period		Ordinal day					Median date	Interquartile range (days)
			Skamania	Summer	Median	1st quartile	3rd quartile	5th percentile	95th percentile		
			Apr. 1-Jun. 30	July 1- Oct. 31							
01_WCOAST	0	0 – 42	0	0	-	-	-	-	-	-	-
02_LOWCOL¶	371*	48 – 948	166	205	-	-	-	-	-	-	-
03_SKAMAN¶	8673*	7150 – 999	4590	4084	173	158	201	115	228	06/22/16	43
04_WILLAM	0	0 – 171	0	0	-	-	-	-	-	-	-
05_BWSALM	0	0 – 38	0	0	-	-	-	-	-	-	-
06_KLICR¶	65	0 – 188	0	65	-	-	-	-	-	-	-
07_MGILCS¶	36137*	32915 – 39603	518	35619	217	203	230	183	258	08/05/16	27
08_YAKIMA	0	0 – 148	0	0	-	-	-	-	-	-	-
09_UPPCOL¶	7084*	5328 – 8988	145	6940	216	203	237	181	257	08/04/16	34
10_SFCLWR¶	51452*	48157 – 54575	0	51452	260	248	268	233	279	09/17/16	20
11_UPCLWR	0	0 – 115	0	0	-	-	-	-	-	-	-
12_SFSALM	0	0 – 94	0	0	-	-	-	-	-	-	-
13_MFSALM	0	0 – 137	0	0	-	-	-	-	-	-	-
14_UPSALM¶	38629*	34899 – 42395	298	38331	227	209	249	187	267	08/15/16	40
Total	142411										

40 *Combined with PBT estimated abundance

41 [¶]Run-timing distributions were not estimated for stocks where the combined number of PBT and GSI assignments to reporting group was n<5.

42

43 **Note:** These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on weekly stock proportions and total

44 numbers of steelhead that were observed passing Bonneville Dam at the fish counting window. The run-timing distributions are characterized by ordinal days for the median date,

45 inter-quartile range (days), and 5th and 95th percentile. The distributions were based on the weekly estimated reporting group proportions that were applied to the daily tallies of

46 steelhead at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Run-timing

47 distributions for hatchery-origin fish include stock abundance estimated from PBT and GSI assignments.

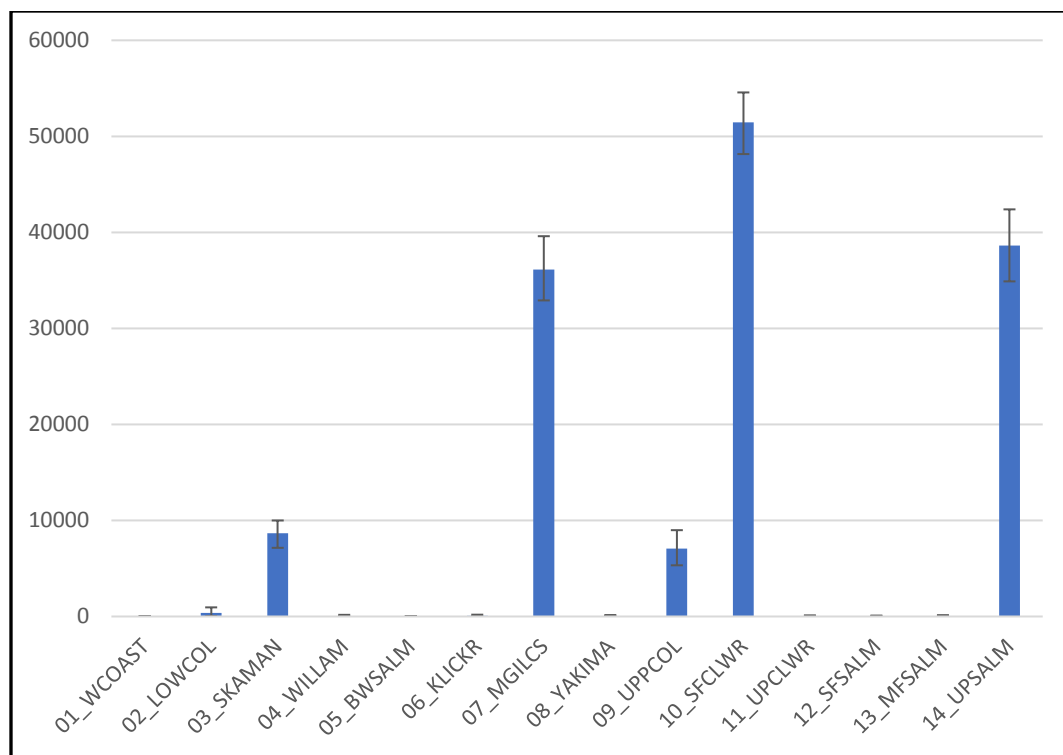


Figure 6: Relative abundance (\pm 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2016.

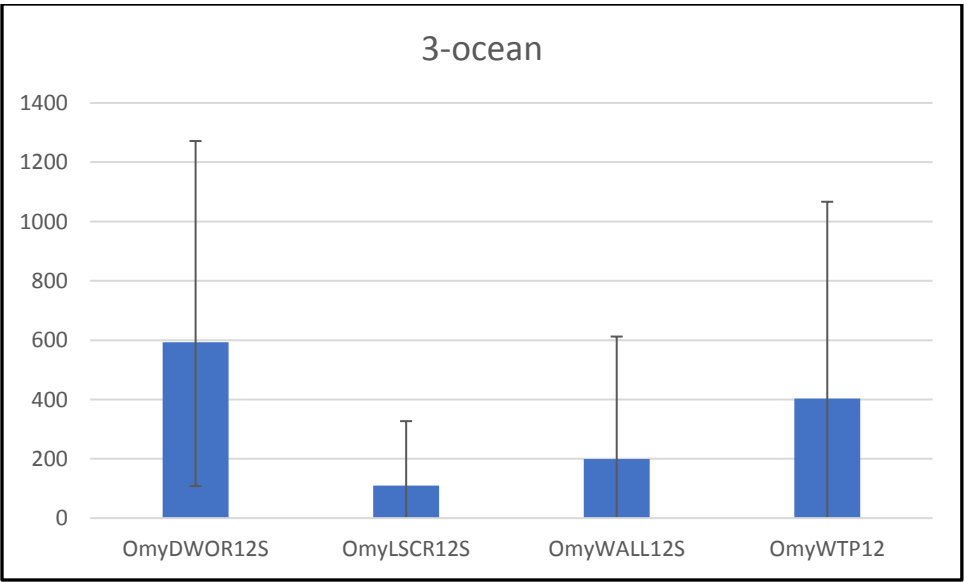
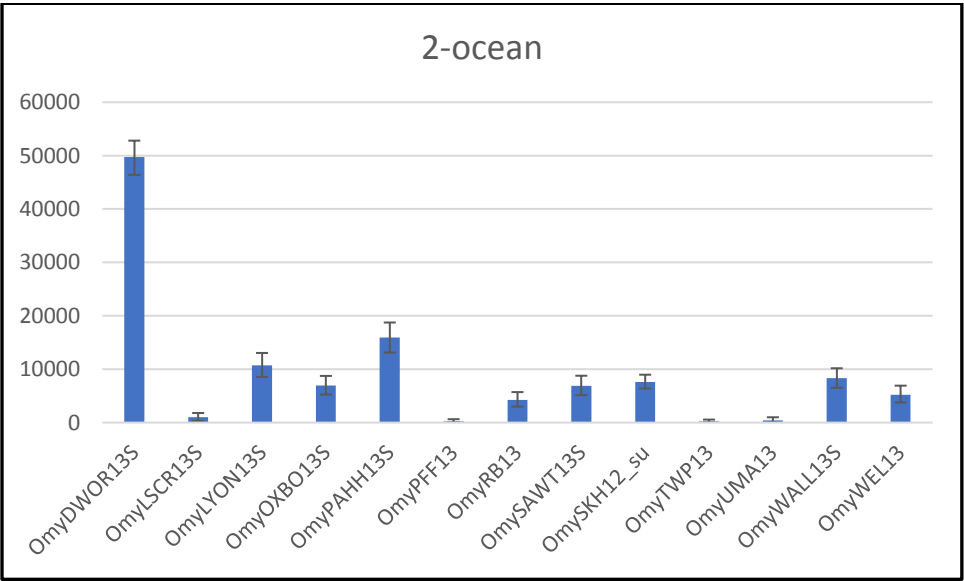
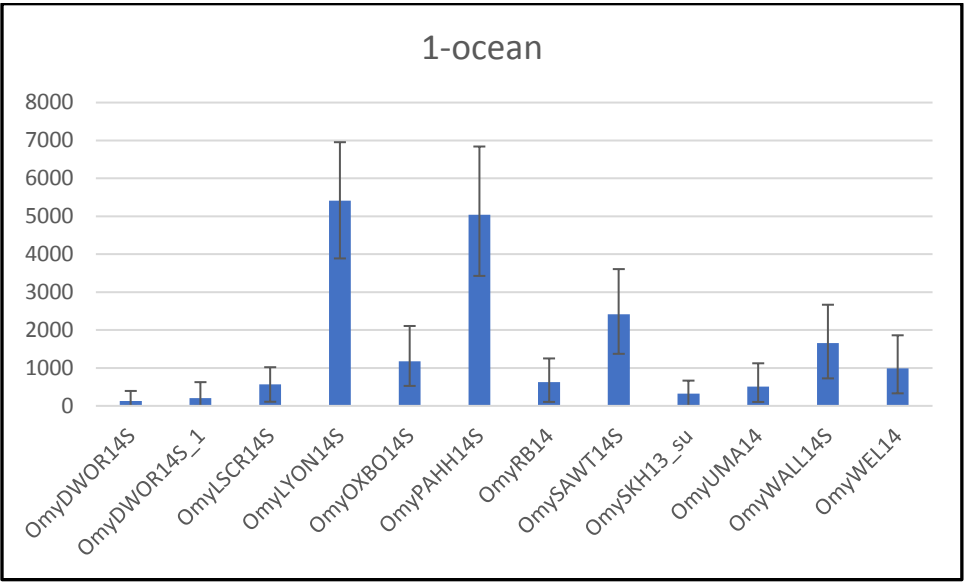
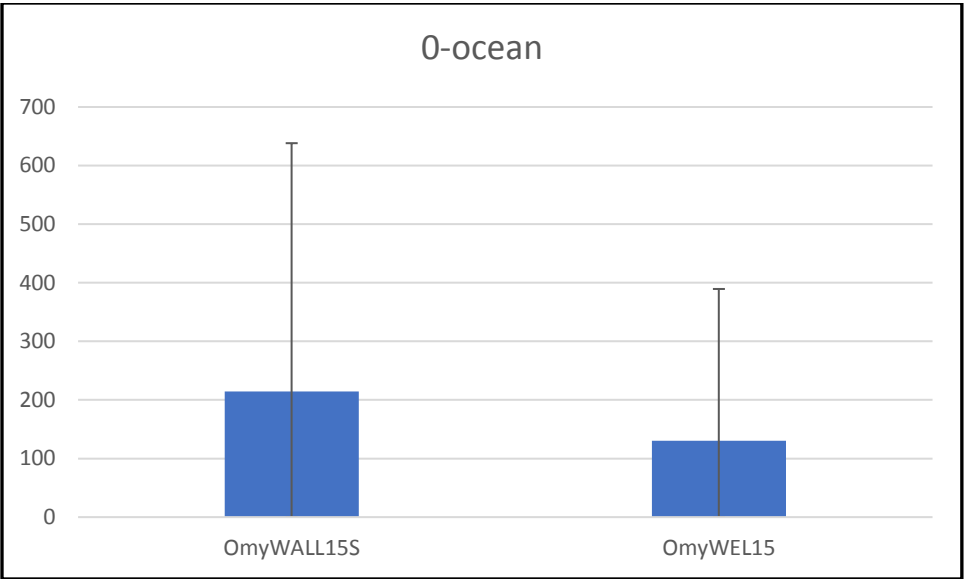


Figure 7: Relative abundance (\pm 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) sampled at Bonneville Dam in 2016 that assigned via PBT to 31 hatchery broodstocks of origin. The 2015 age-class (0-ocean fish; top panel), 2014 age-class (1-ocean fish), 2013 age class (2-ocean fish), and 2012 age class (3-ocean fish) are shown. Key to broodstock collection is presented in Section 3 (Table 1). Skamania broodstock are spawned earlier in the year and are designated for the following brood year.

61 Table 10: Hatchery broodstock-specific relative abundance and run-timing distributions of adipose clipped and non-clipped PBT-assigned steelhead passing Bonneville Dam in 2016. Key to broodstock
62 collection is presented in Section 3 (Table 3). Skamania broodstock are spawned earlier in the year and are designated for the following brood year.

Reporting Group	Broodstock collection	Tagging rate	Estimated abundance				Run-timing distribution						
			Mean	95% CI	Management Period		Ordinal day					Median	Interquartile
					Skamania	Summer	Median	1st	3rd	5th	95th		
					Apr. 1-Jun. 30	July 1- Oct. 31		quartile	quartile	percentile	percentile		
02_LOWCOL	OmyPFF13*	95.0%	205	0 – 638	0	205	-	-	-	-	-	-	-
03_SKAMAN	OmySKH12_su ^a	99.0%	7583	6386 – 8971	4178	3405	173	158	201	116	228	06/22/16	43
	OmySKH13_su ^{b*}	100.0%	320	0 – 666	0	320	-	-	-	-	-	-	-
07_MGILCS	OmyLSCR12S*	95.6%	110	0 – 327	0	110	-	-	-	-	-	-	-
	OmyLSCR13S	72.8%	1013	299 – 1799	0	1013	207	196	219	182	230	07/26/16	23
	OmyLSCR14S	95.5%	564	109 – 1020	0	564	213	205	223	187	230	08/01/16	18
	OmyLYON13S	100.0%	10704	8555 – 13042	0	10704	222	205	243	186	258	08/10/16	38
	OmyLYON14S	99.1%	5411	3889 – 6955	0	5411	221	210	232	196	263	08/09/16	22
	OmyRB13	98.0%	4260	2989 – 5714	297	3963	208	196	223	171	252	07/27/16	27
	OmyRB14	99.0%	626	105 – 1252	0	626	221	207	238	188	257	08/09/16	31
	OmyUMA13*	94.0%	437	0 – 996	0	437	-	-	-	-	-	-	-
	OmyUMA14*	100.0%	510	103 – 1124	0	510	-	-	-	-	-	-	-
	OmyWALL12S*	99.2%	199	0 – 612	0	199	-	-	-	-	-	-	-
	OmyWALL13S	91.0%	8313	6518 – 10176	196	8117	212	200	225	181	260	07/31/16	25
	OmyWALL14S	99.6%	1659	725 – 2668	0	1659	231	218	248	206	258	08/19/16	30
	OmyWALL15S*	95.1%	214	0 – 638	0	214	-	-	-	-	-	-	-
	09_UPPCOL	OmyTWP13*	100.0%	221	0 – 567	0	221	-	-	-	-	-	-
OmyWEL13		99.0%	5230	3759 – 6920	145	5085	215	199	233	180	257	08/03/16	34
OmyWEL14		97.0%	993	331 – 1862	0	993	224	199	247	183	257	08/12/16	48
OmyWEL15*		98.0%	131	0 – 389	0	131	-	-	-	-	-	-	-
OmyWTP12*		78.0%	404	0 – 1067	0	404	-	-	-	-	-	-	-
10_SFCLWR	OmyDWOR12S*	96.3%	593	108 – 1272	0	593	-	-	-	-	-	-	-
	OmyDWOR13S	96.7%	49712	46402 – 52806	0	49712	259	248	268	233	279	09/16/16	20
	OmyDWOR14S*	97.2%	128	0 – 394	0	128	-	-	-	-	-	-	-
	OmyDWOR14S_1*	96.9%	201	0 – 626	0	201	-	-	-	-	-	-	-
14_UPSALM	OmyOXBO13S	97.1%	6936	5257 – 8743	0	6936	218	206	231	187	257	08/06/16	25
	OmyOXBO14S	97.0%	1176	527 – 2108	0	1176	226	214	244	206	257	08/14/16	30
	OmyPAHH13S	98.6%	15956	13117 – 18746	141	15816	231	208	253	186	270	08/19/16	45
	OmyPAHH14S	99.7%	5039	3428 – 6841	0	5039	233	215	251	196	260	08/21/16	36
	OmySAWT13S	99.3%	6888	5153 – 8778	157	6731	226	206	250	183	269	08/14/16	44
	OmySAWT14S	99.4%	2414	1373 – 3607	0	2414	230	215	249	204	260	08/18/16	34
Total			138150		5114	133036							

63 *Run-timing distributions were not estimated for hatchery broodstocks where the number of PBT assignments was n<5.

64 ^a OmySKH12_su are 2-ocean

65 ^b OmySKH13_su are 1-ocean

66

67 **Note:** These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on stock proportions and total numbers of steelhead that were observed
68 passing Bonneville Dam at the fish counting window. The date ranges listed under “Skamania” and “Summer” management period were chosen by steelhead fishery managers, and for each hatchery source
69 stock we provide the abundance that has passed within these time periods.

70 Table 11: Relative abundance and run-timing distributions of natural origin (adipose non-clipped) steelhead stocks passing Bonneville Dam in 2016.

Reporting Group	Estimated abundance				Run-timing distribution						
	Mean	95% CI	Management Period		Ordinal day					Median date	Interquartile range (days)
			Skamania	Summer	Median	1st	3rd	5th	95th		
			Apr. 1-Jun. 30	July 1- Oct. 31		quartile	quartile	percentile	percentile		
01_WCOAST	0	0 – 77	0	0	-	-	-	-	-	-	-
02_LOWCOL	1015	320 – 1521	436	578	197	168	222	143	253	07/16/16	54
03_SKAMAN	623	273 – 1447	542	81	166	152	173	129	219	06/15/16	21
04_WILLAM	0	0 – 358	0	0	-	-	-	-	-	-	-
05_BWSALM*	287	20 – 754	0	287	-	-	-	-	-	-	-
06_KLICKR	2372	1607 – 3706	712	1661	192	173	205	151	240	07/11/16	32
07_MGILCS	25335	25016 – 29269	570	24765	207	194	223	179	255	07/26/16	29
08_YAKIMA	2003	947 – 2826	0	2003	206	195	220	181	268	07/25/16	25
09_UPPCOL*	1133	0 – 1475	208	926	-	-	-	-	-	-	-
10_SFCLWR	334	86 – 928	0	334	268	264	275	260	284	09/25/16	11
11_UPCLWR	2300	1273 – 3076	194	2107	253	239	264	170	278	09/10/16	25
12_SFSALM*	436	84 – 1145	0	436	-	-	-	-	-	-	-
13_MFSALM	1443	538 – 2237	194	1249	227	193	253	165	272	08/15/16	60
14_UPSALM	1937	3 – 2457	191	1746	234	209	248	168	258	08/22/16	39
Total	39218										

71 * Run-timing distributions were not estimated for stocks where the number of GSI assignments was n<5.

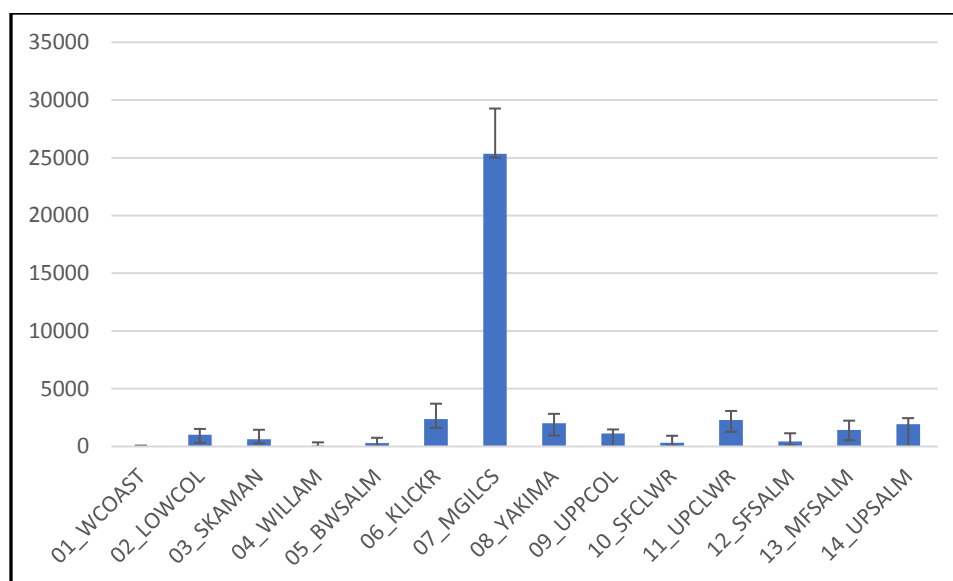


Figure 8: Relative abundance (\pm 95% CI) of natural origin (adipose non-clipped) steelhead sampled at Bonneville Dam in 2016 assigned to genetic stock of origin.

Run-timing of steelhead stocks in 2016

We were able to characterize the run-timing distributions for five hatchery origin steelhead stocks (Table 9; Figure 9). Very few winter-run steelhead stocks exist above Bonneville Dam and our sampling program at Bonneville AFF does not trap or collect fish between December and March when winter-run steelhead would be most likely to occur. Thus, winter-run stocks are expected to be rare in our samples from Bonneville Dam, but could occur from hatchery-origin fish from PFF in the Hood River (winter-run broodstock) or natural-origin fish from sub-basins nearest upstream such as Klickitat, Hood, and Fifteenmile rivers. The 03_SKAMAN hatchery stock has previously been characterized as part of an early run-timing category, and in 2016 had a median run-timing date of 6/22/2016 (ordinal day 173). An intermediate run-timing category has also been described, and includes the following steelhead stocks (ordered by median date): 09_UPPCOL 8/4/16; ordinal day 216), 07_MGILCS (8/5/16; ordinal day 217), and 14_UPSALM (8/15/16; ordinal day 227). Finally, a late run-timing category consists of the 10_SFCLWR stock (9/17/16; ordinal day 260), and is typically thought to be characteristic of B-run steelhead that return after August 25th at Bonneville Dam (Table 9; Figure 9).

Using the PBT-assigned steelhead, we also characterized the run-timing distributions for major broodstock sources (mean estimated abundance \geq 1000 fish) in our PBT baseline (Table 10; Figure 10). For this analysis, we grouped the 15 major hatchery broodstock sources (abundance >1000) into categories according to their median run-timing date. The following four broodstock sources had a median run-timing date before August 1st: OmySKH12_SU, OmyLSCR13S, OmyRB13, and OmyWALL13S. Ten broodstock sources had median run-timing dates from August 1 – August 29 (i.e., OmyWEL13, OmyOXBO13S, OmyOXBO14S, OmyLYON13S, OmyLYON14S, OmyPAHH13S, OmyPAHH14S, OmySAWT13S, OmySAWT14S, and OmyWALL14S). Only OmyDWOR13S had median run-timing distributions after September 1st (Table 10).

We were able to characterize the run-timing distributions for nine natural-origin steelhead stocks (Table 11; Figure 9); patterns generally fit the same run-timing categories as characterized for hatchery-origin steelhead. The nine natural origin stocks ordered by median run-timing date

were: 03_SKAMAN (6/15/16; ordinal day 166), 06_KLICKR (7/11/16; ordinal day 192), 02_LOWCOL (7/16/6; ordinal day 197), 08_YAKIMA (7/25/16; ordinal day 206), 07_MGILCS (7/26/16; ordinal day 207), 13_MFSALM (8/15/15; ordinal day 227), 14_UPSALM (8/22/15; ordinal day 234), 11_UPCLWR (9/10/16; ordinal day 253), and 10_SFCLWR (9/25/16; ordinal day 268) (Table 11; Figure 9).

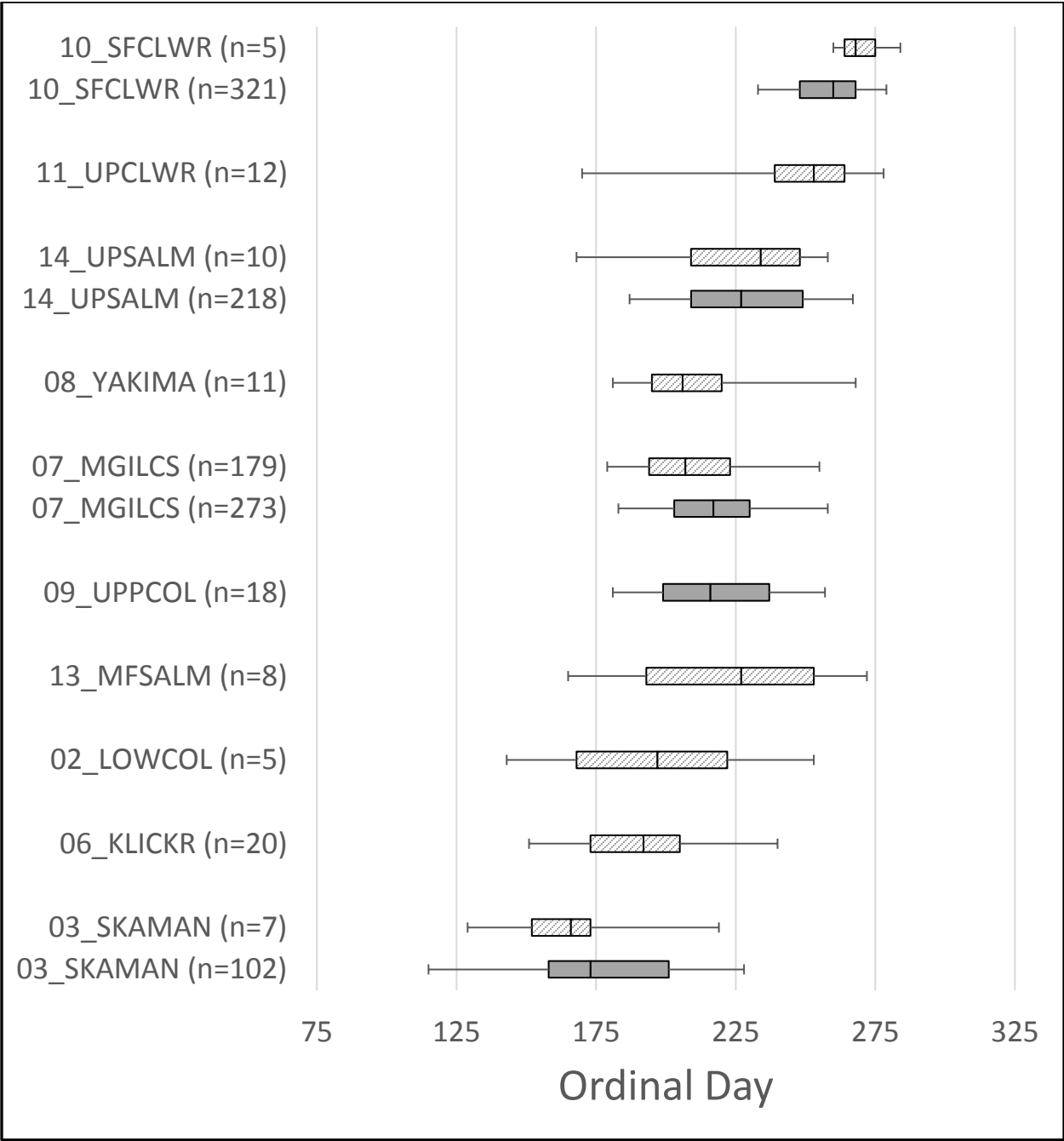
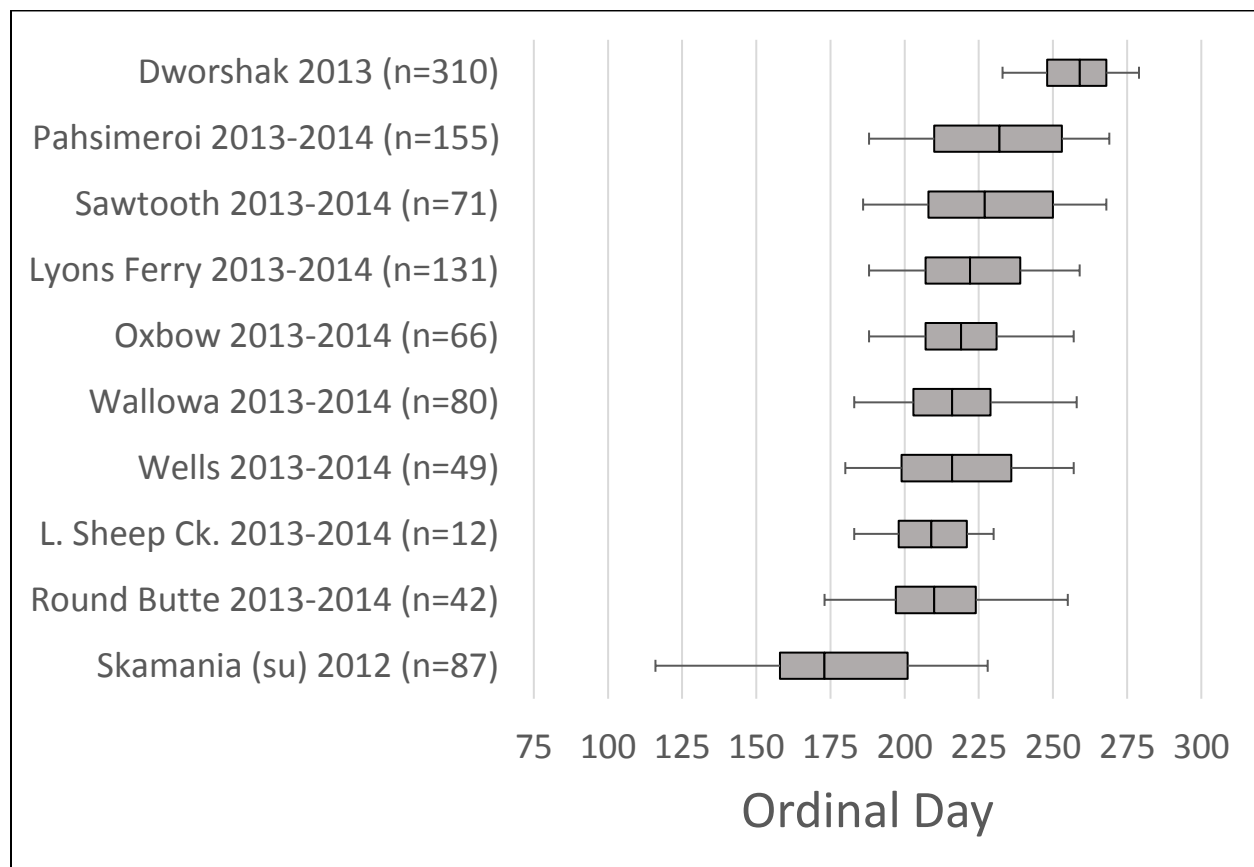


Figure 9: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin steelhead (adipose clipped and non-clipped; solid filled) and natural origin steelhead (adipose non-clipped; hash-marked) that were sampled at Bonneville Dam in 2016 and assigned to stock of origin. The number of PBT and/or GSI assignments used in the relative stock abundance expansion procedure from which these run-timing distributions are derived are shown.



119

120 Figure 10: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th
 121 percentile) for hatchery origin steelhead (adipose clipped and non-clipped) assigned to PBT
 122 broodstock of origin that were sampled at Bonneville Dam in 2016. Hatcheries and their
 123 broodyears are ordered from earliest to latest run-timing. Key to broodstock collection is
 124 presented in Section 3 (Table 3). The number of PBT assignments used in the relative stock
 125 abundance expansion procedure from which these run-timing distributions are derived are
 126 shown.

PBT and GSI assignments for steelhead in 2016

We were able to assign 1028 steelhead sampled at Bonneville Dam in 2016 to 31 hatchery broodstock sources (2012-2015) throughout the Columbia River basin (Table 10,12). The largest portion of the PBT-assigned fish originated from the Dworshak Hatchery (n=316; 31%), followed by Pahsimeroi (n=155; 15%), Lyons Ferry (n=131; 13%), Skamania (n=90; 9%), and Wallowa (n=82; 8%) hatcheries. Using these known hatchery-of-origin steelhead, we compared the individual assignments from GSI analysis, and used these assignments to help classify them into reporting groups (Table 12). Those groupings were used to combine results of PBT-hatchery abundance estimates with the GSI estimated abundance of hatchery stocks (Table 10). Tagging rates varied across source hatcheries from 72.8% (OmyLSCR13S) to 100% (OmyLYON13S, OmyTWP13, OmySKH13_su, and OmyUMA14) (Table 10,12).

Using GSI, we were able to assign 264 natural-origin and 57 hatchery-origin steelhead (that could not be assigned via PBT) sampled at Bonneville Dam in 2016 to 14 reporting groups throughout the Columbia River basin (Table 13). The majority (91%) of these assignments were to the inland lineage (dominated by assignments to 07_MGILCS; 70%); 9% assigned to the coastal lineage (dominated by assignments to 03_SKAMAN; 68%) (Table 13).

We examined which of the hatchery sources contributed to the size range of fish typically classified as B-run steelhead (Table 14). Fish with a fork length ≥ 78 cm were found to primarily originate from the Dworshak broodstock sources, and most of these were from the 2013 (2-ocean age) spawn year (Table 14). The 2-ocean age is typical of B-run life history. Further, the regions of the South Fork and Upper Clearwater R. and Middle Fork and South Fork Salmon R. are generally thought to be the largest sources of B-run steelhead. Dworshak broodstock fit within the South Fork Clearwater R. genetic stock and so are expected to produce large, older steelhead.

150 Table 12: Summary information about the PBT steelhead hatchery broodstock sources and
 151 number of assignments. Key to broodstock collection is presented in Section 3 (Table 3).
 152 Skamania broodstock are spawned earlier in the year and are designated for the following brood
 153 year.

Broodstock collection	Basin	Reporting Group	Tagging rate (%)	Age	Adipose fin		Total
					Clipped	Non-clipped	
OmyPFF13	Columbia	02_LOWCOL	0.9500	2-ocean	1		1
OmySKH12_su	Columbia	03_SKAMANIA	0.9900	3-ocean	85	2	87
OmySKH13_su	Columbia	03_SKAMANIA	1.0000	2-ocean	3		3
OmyLSCR12S	Snake	07_MGILCS	0.9557	3-ocean	1		1
OmyLSCR13S	Snake	07_MGILCS	0.7277	2-ocean	7		7
OmyLSCR14S	Snake	07_MGILCS	0.9550	1-ocean	5		5
OmyLYON13S	Snake	07_MGILCS	1.0000	2-ocean	82	3	85
OmyLYON14S	Snake	07_MGILCS	0.9939	1-ocean	44	2	46
OmyRB13	Columbia	07_MGILCS	0.9800	2-ocean	33	4	37
OmyRB14	Columbia	07_MGILCS	0.9900	1-ocean	5		5
OmyUMA13	Columbia	07_MGILCS	0.9400	2-ocean	3		3
OmyUMA14	Columbia	07_MGILCS	1.0000	1-ocean	4		4
OmyWALL12S	Snake	07_MGILCS	0.9918	3-ocean	1		1
OmyWALL13S	Snake	07_MGILCS	0.9102	2-ocean	65	3	68
OmyWALL14S	Snake	07_MGILCS	0.9959	1-ocean	12		12
OmyWALL15S	Snake	07_MGILCS	0.9510	0-ocean	1		1
OmyTWP13	Columbia	09_UPPCOL	1.0000	2-ocean		2	2
OmyWEL13	Columbia	09_UPPCOL	0.9900	2-ocean	41	1	42
OmyWEL14	Columbia	09_UPPCOL	0.9700	1-ocean	6	1	7
OmyWEL15	Columbia	09_UPPCOL	0.9800	0-ocean	1		1
OmyWTP12	Columbia	09_UPPCOL	0.7800	3-ocean	2		2
OmyDWOR12S	Snake	10_SFCLWR	0.9614	3-ocean	2	2	4
OmyDWOR13S	Snake	10_SFCLWR	0.9642	2-ocean	252	58	310
OmyDWOR14S	Snake	10_SFCLWR	0.9689	1-ocean	1		1
OmyDWOR14S_1	Snake	10_SFCLWR	0.9689	1-ocean	1		1
OmyOXBO13S	Snake	14_UPSALM	0.9709	2-ocean	56	1	57
OmyOXBO14S	Snake	14_UPSALM	0.9701	1-ocean	9		9
OmyPAHH13S	Snake	14_UPSALM	0.9944	2-ocean	108	11	119
OmyPAHH14S	Snake	14_UPSALM	0.9962	1-ocean	36		36
OmySAWT13S	Snake	14_UPSALM	0.9965	2-ocean	46	7	53
OmySAWT14S	Snake	14_UPSALM	0.9942	1-ocean	17	1	18

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155 Table 13: Summary of the number of GSI assignments by reporting group for hatchery-origin
 156 steelhead that did not assign via PBT, and natural-origin steelhead that assigned via GSI.

Reporting group	Lineage	Adipose fin		Total
		Clipped	Non-clipped	
01_WCOAST	Coastal			
02_LOWCOL	Coastal	2	5	7
03_SKAMAN	Coastal	12	7	19
04_WILLAM	Coastal			
05_BWSALM	Coastal		2	2
06_KLICKR	Inland		20	20
07_MGILCS	Inland	27	179	206
08_YAKIMA	Inland		11	11
09_UPPCOL	Inland		2	2
10_SFCLWR	Inland	14	5	19
11_UPCLWR	Inland		12	12
12_SFSALM	Inland		3	3
13_MFSALM	Inland		8	8
14_UPSALM	Inland	2	10	12
	Total	57	264	321

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Table 14: Summary information for Columbia River and Snake River hatchery broodstock sources for steelhead sampled at Bonneville Dam in 2016. The percent of steelhead that were identified to be B-run fish (i.e., ≥ 78 cm fork length) by sex from each broodstock are shown. Skamania broodstock are spawned earlier in the year and are designated for the following brood year.

Reporting Group	Broodstock collection	Tagging rate	Age	Sex and size				
				Females		Males		Total
				N	%B-run (≥ 78 cm FL)	N	%B-run (≥ 78 cm FL)	N
02_LOWCOL	OmyPFF13	95.0%	2-ocean	1	0.0%	0	0.0%	1
03_SKAMANIA	OmySKH12_su	99.0%	2-ocean	13	0.0%	74	1.4%	87
	OmySKH13_su	100.0%	1-ocean	0	0.0%	3	0.0%	3
07_MGILCS	OmyLSCR12S	95.6%	3-ocean	0	0.0%	1	0.0%	1
	OmyLSCR13S	72.8%	2-ocean	4	0.0%	3	0.0%	7
	OmyLSCR14S	95.5%	1-ocean	1	0.0%	4	0.0%	5
	OmyLYON13S	100.0%	2-ocean	52	0.0%	33	0.0%	85
	OmyLYON14S	99.1%	1-ocean	23	0.0%	23	0.0%	46
	OmyRB13	98.0%	2-ocean	24	0.0%	13	0.0%	37
	OmyRB14	99.0%	1-ocean	1	0.0%	4	0.0%	5
	OmyUMA13	94.0%	2-ocean	1	0.0%	2	0.0%	3
	OmyUMA14	100.0%	1-ocean	3	0.0%	1	0.0%	4
	OmyWALL12S	99.2%	3-ocean	1	0.0%	0	0.0%	1
	OmyWALL13S	91.0%	2-ocean	26	0.0%	42	0.0%	68
	OmyWALL14S	99.6%	1-ocean	6	0.0%	6	0.0%	12
	OmyWALL15S	95.1%	0-ocean	0	0.0%	1	0.0%	1
09_UPPCOL	OmyTWP13	100.0%	2-ocean	1	0.0%	1	0.0%	2
	OmyWEL13	99.0%	2-ocean	19	0.0%	23	4.4%	42
	OmyWEL14	97.0%	1-ocean	3	0.0%	4	0.0%	7
	OmyWEL15	98.0%	0-ocean	0	0.0%	1	0.0%	1
	OmyWTP12	78.0%	3-ocean	2	0.0%	0	0.0%	2
10_SFCLWR	OmyDWOR12S	96.3%	3-ocean	1	100.0%	3	66.6%	4
	OmyDWOR13S	96.7%	2-ocean	119	71.4%	191	81.2%	310
	OmyDWOR14S	97.2%	1-ocean	0	0.0%	1	0.0%	1
	OmyDWOR14S_1	96.9%	1-ocean	0	0.0%	1	0.0%	1
14_UPSALM	OmyOXBO13S	97.1%	2-ocean	25	0.0%	32	0.0%	57
	OmyOXBO14S	97.0%	1-ocean	5	0.0%	4	0.0%	9
	OmyPAHH13S	98.6%	2-ocean	56	0.0%	62	8.1%	118
	OmyPAHH14S	99.7%	1-ocean	24	0.0%	12	0.0%	36
	OmySAWT13S	99.3%	2-ocean	34	0.0%	19	5.3%	53
	OmySAWT14S	99.4%	1-ocean	12	0.0%	6	0.0%	18

Estimated relative abundance and run-timing of sockeye salmon stocks in 2016

Relative stock abundance for sockeye salmon was estimated over a course of 11 statistical weeks (i.e. weeks 22-33) and were grouped to obtain a minimum of n=20 per group. A total of 1686 sockeye salmon were sampled at Bonneville Dam in 2016 and were assigned to one of five genetic stocks (i.e., Okanogan, Wenatchee, Snake, Lake Billy Chinook, and Wallowa) (Table 15). Here, the Wallowa stock is characterized by kokanee. The Okanogan stock had the highest relative abundance (240,163), followed by the Wenatchee (99,037) (Figure 11). The Snake, Lake Billy Chinook, and Wallowa stocks all had estimated abundances < 1500, but were based on relatively few genetic assignments (<15) (Table 15).

We characterized the run-timing distributions for four sockeye salmon stocks (Table 15, Figure 12). The Okanogan and Wenatchee stocks had nearly identical run timing distributions (median date: 6/21/16 and 6/22/16, respectively). The median run-timing for the Snake and Lake Billy Chinook stocks occurred on 6/29/16 and 7/11/16, respectively (Figure 1).

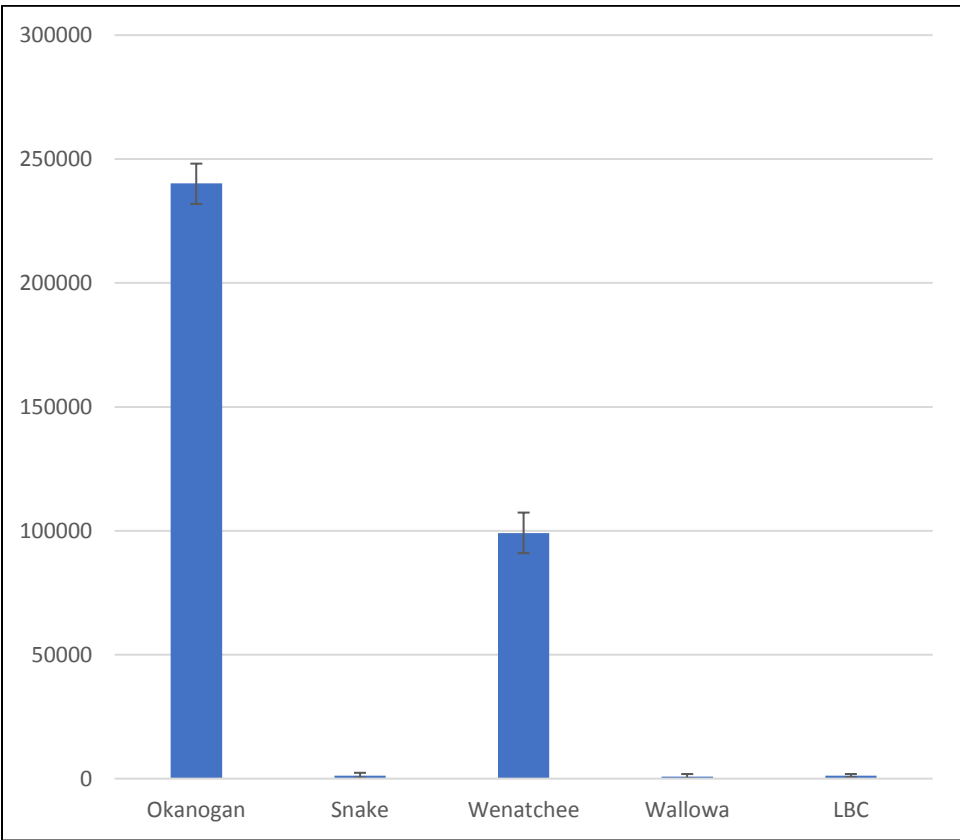


Figure 11: Relative abundance (± 95% CI) of sockeye salmon stocks sampled at Bonneville Dam in 2016.

182 Table 15: Relative abundance and run-timing distributions of sockeye salmon stocks passing Bonneville Dam in 2016.

Reporting group	N	Estimated abundance		Run -timing distributions						
				Ordinal day					Median date	Interquartile range
		Mean	95% CI	Median	1st quartile	3rd quartile	5th percentile	95th percentile		
Okanogan	1167	240163	231,882 - 248,158	172	168	177	161	188	06/21/16	9
Wenatchee	495	99037	90,961 - 107,348	173	168	178	162	192	06/22/16	10
Snake	7	1202	375 - 2,348	180	178	185	176	212	06/29/16	7
Lake Billy Chinook	13	799	556 – 1,853	192	186	199	183	212	07/11/16	13
Wallowa*	4	1160	72 – 1,857	-	-	-	-	-	-	-
Total	1687	342361								

183 * Run-timing distributions were not estimated for stocks where the number of GSI assignments was n<5

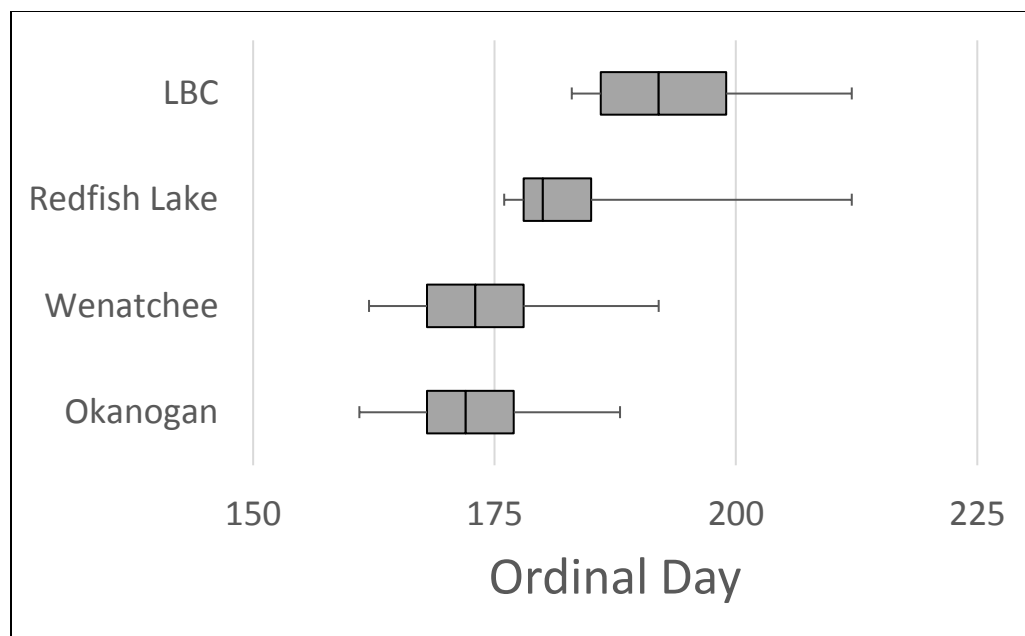


Figure 12: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for sockeye salmon that were sampled at Bonneville Dam in 2016 and assigned to stock of origin.

Future directions

The delayed migratory run-timing for Chinook salmon at Bonneville Dam during the spring of 2017 prompted an ‘in-season’ assessment of stock composition. We were interested in the feasibility of providing timely results to fisheries managers, and examined the stock composition and relative stock abundances for adult spring Chinook (i.e. ≥ 58 cm FL) for the following time periods i) 01/01/17 – 05/31/17, ii) 06/01/17 – 06/15/17, and iii) 06/16/17 – 06/30/17. For each time period, we were able to provide results to managers within approximately two weeks of the end of sampling; this included time for shipping of samples, laboratory processing, and data analysis – a marked improvement over previous years. Results indicated that the three largest components of hatchery-origin fish of interior stream-type lineage (spring/summer run fish) passing Bonneville Dam over the course of this assessment was comprised of the 12_HELLSC (43,450), 20_BONPOOLSP (19,293), and 21_UMATILLASP (12,732) hatchery stocks (Table 16). Since the sampling period through 6/30/17 included returns of interior ocean-type (summer/fall run fish), we also detected relatively large numbers of hatchery origin fish from the 18_UCOLSF (20,826) stock. The three largest components of natural-origin fish of interior stream-type lineage (spring/summer run fish) during the in-season assessment was comprised of the 09_YAKIMA (2,203), and 12_HELLSC (1,595), and 16_UPSALM (1,288) genetic stocks (Table 17). Again, since the sampling period through 6/30/17 included returns of interior ocean-type (summer/fall run fish), we also detected relatively large numbers of natural origin fish from the 18_UCOLSF (15,337) genetic stock.

Returning Chinook salmon in 2017 provided an opportunity to report PBT assignments by specific locations of hatcheries rather than previous reporting groups that were not necessarily

geographically accurate due to genetic similarity for GSI assignments. This will provide much better representation of spring Chinook returning to the upper Columbia now that fish from Carson Hatchery and Umatilla Hatchery can be accurately identified with PBT and split into their own reporting groups (Carson Hatchery=20_BONPOOLSP; Umatilla Hatchery=21_UMATILLASP). Similarly, fall Chinook salmon that had previously been part of the 18_UPCOLSF reporting group due to genetic similarity but were geographically inaccurate, will now be assigned to more representative locations including hatchery origin fall Chinook from Little White Salmon Hatchery(22_BONPOOLFA) and Umatilla Hatchery (23_UMATILLAFA). Assignment of natural origin Chinook salmon will continue to be assigned with GSI methods to original reporting groups with expected accuracy levels reported in Table 9 in Section 3. However, further SNP markers that strongly distinguish various natural stocks of Chinook salmon are in development that should lead to dramatic improvement in accuracy of GSI.

Returning steelhead in 2017 will also see changes to provide more informative stock ID results, in particular for steelhead returning to the mid-Columbia and lower Snake that have been previously lumped into a large reporting group known as 07_MGILCS (see Section 3 for reporting group details). Additional SNP markers have been identified that better distinguish steelhead in the 07_MGILCS group and will allow assignments to more relevant reporting groups.

Having demonstrated the feasibility of in-season estimates of stock composition and relative abundance for spring Chinook returning in 2017, we plan to implement in-season estimates for all stocks in 2018. This includes improved efficiencies by integrating the entire analytical process (i.e., stock composition, relative abundance estimation, run-timing distribution) into an R framework that standardizes and streamlines analyses. The development of this tool has greatly decreased the amount of time required to estimate various components of the analysis, and we anticipate that the annual report for 2017 (covering the period 1/1/2018-12/31/2018) will included results for both calendar years 2017 and 2018.

238 Table 16: Stock-specific relative abundance of hatchery origin (adipose clipped and non-clipped)
 239 adult (≥ 58 cm FL) spring Chinook salmon passing Bonneville Dam during the period 01/01/17–
 240 06/30/17.

Reporting Group	Estimated abundance	
	Mean	95% CI
01_YOUNGS	0	0 – 139
02_WCASSP	28	3 – 231
03_WCASFA	0	1 – 335
04_WILLAM	58	7 – 328
05_SPCRTU	0	0 – 204
06_KLICKR	2744	1674 – 4014
07_DESCSP	9377	7018 – 11547
08_JOHNDR	384	26 – 731
09_YAKIMA	1604	932 – 2439
10_UCOLSP	3827	2654 – 5197
11_TUCANO	690	174 – 1367
12_HELLSC	43450	40047 – 46621
13_SFSALM	2171	1063 – 4203
14_CHMBLN	0	0 – 129
15_MFSALM	545	28 – 826
16_UPSALM	1645	973 – 2807
17_DESCFA	0	0 – 128
18_UCOLSF	20826	18694 – 22184
19_SRFALL	277	4 – 747
20_BONPOOLSP	19293	16924 – 22156
21_UMATILLASP	12732	10568 – 14897
22_BONPOOLFA	0	0 – 0
23_UMATILLAFA	0	0 – 0
Total	119651	

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Table 17: Stock-specific relative abundance of natural origin (adipose non-clipped) adult (≥ 58 cm FL) spring Chinook salmon passing Bonneville Dam during the period 01/01/17– 06/30/17.

Reporting Group	Estimated abundance	
	Mean	95% CI
01_YOUNGS	0	0 – 162
02_WCASSP	164	2 – 637
03_WCASFA	132	17 – 622
04_WILLAM	59	8 – 389
05_SPCRTU	0	0 – 244
06_KLICKR	378	58 – 1040
07_DESCSP	122	1 – 377
08_JOHNDR	1133	95 – 1697
09_YAKIMA	2203	1263 – 3040
10_UCOLSP	1049	366 – 2093
11_TUCANO	0	0 – 165
12_HELLSC	1595	976 – 3698
13_SFSALM	1177	434 – 2357
14_CHMBLN	0	0 – 174
15_MFSALM	664	223 – 1479
16_UPSALM	1288	593 – 2228
17_DESCFA	0	0 – 168
18_UCOLSF	15337	13786 – 15582
19_SRFALL	48	0 – 431
Total	25349	

Discussion

Parentage based tagging (PBT) and genetic stock identification (GSI) may be considered as methods that could replace the central functions of the coded wire tag program and could be a replacement for adipose fin marking to identify hatchery origin fish. However, this replacement would be contingent on continued genotyping of hatchery broodstock, fish passing Bonneville Dam, and harvested fish. For ocean fisheries management, additional hatcheries throughout the range of Chinook salmon would have to contribute broodstock samples to this PBT baseline in order for the method to serve ocean fisheries management and the need to monitor total fishery impacts for stocks including Columbia River stocks of fall Chinook (tules and upriver brights) harvested in ocean fisheries. The genetic methods provide a substantial amount of information when they are combined and used to analyze Columbia River Chinook salmon and steelhead passing Bonneville Dam. PBT improves the accuracy for defining hatchery-origin and by subtraction, total natural-origin stocks. Expansion of our PBT baseline to include hatcheries in the Columbia River has increased the proportion of hatchery origin fish passing Bonneville Dam that can be assigned to their broodstock source. For instance, we were able to assign 59% of all

steelhead sampled at Bonneville Dam in the previous year of 2015 to 20 broodstock sources throughout the Columbia River basin. This increased to 80% for all steelhead sampled at Bonneville Dam in 2016, where we were able to assign hatchery origin fish to 31 broodstock sources due to the sampling of Columbia River hatcheries from 2012-2015. As this effort continues to expand, we anticipate a corresponding increase in the proportion of hatchery origin fish that can be confidently assigned to their hatchery broodstock source.

Genetic monitoring combining PBT and GSI is one of a number of possible tools that can be used to identify hatchery and natural fish at various resolutions. Other methods include, CWTs, PIT tags, VIE tags, and otolith marks. Adipose fin clips can be used to differentiate hatchery fish from wild fish either when fish are clipped at 100% or through expansions if stocks are not clipped at 100%. PBT can further discriminate among hatchery stocks within the reporting groups that we use for GSI analyses, and so we can now characterize different age-classes from particular hatcheries by run-timing distributions and estimate their abundance at Bonneville Dam. GSI continues to provide information that would not be possible with PBT, especially for natural-origin stocks.

This long-term study will allow us to characterize trends in run timing and abundance of steelhead and Chinook and sockeye salmon and provide this data to fisheries managers. We were able to address the following **F&W Program Management Questions:**

What are the status and trend of adult productivity of fish populations?

What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?

Trapping at Bonneville Dam can only be done at very low rates due to restrictions placed on trap operations by USACE and NFMS. Low sample rates inhibit getting a representative sample of various stocks of fish. Higher sample rates would improve the precision of the estimates of fish at Bonneville Dam. Some fisheries were also sampled at very low rates.

We identified 11 Chinook salmon hatchery-origin stocks (61 hatchery broodstock sources) and 12 natural-origin stocks estimated to have relative abundances $\geq 1,000$ fish passing Bonneville Dam in 2016. It may interest fisheries managers to know that the run-timing of various hatchery- and natural-origin 'spring-run' stocks (i.e., 06_KLICKR, 09_YAKIMA, and 13_SFSALM) contributed to the total abundance of adult and jack Chinook salmon that pass through the Columbia River mainstem in two management periods (i.e., spring and summer).

We identified five steelhead hatchery-origin stocks (31 hatchery broodstock sources) and eight natural-origin stocks estimated to have relative abundances $\geq 1,000$ fish passing Bonneville Dam in 2016. We found that genetic stocks seemed to fit well into the historical management categories, particularly the hatchery-origin stocks. Genetic stocks included an early Skamania summer-run, an intermediate run-timing category that contains most natural- and hatchery-origin steelhead stocks, and a late run-timing category with stocks that exhibit median dates after August 25th and includes hatchery- and natural-origin stocks from the South Fork Clearwater River, and the natural-origin stock from upper Clearwater River. Characteristics of steelhead that assigned to Snake River hatchery broodstock sources generally support the typical A-run and B-run steelhead life history categories. The relatively large (≥ 78 cm) steelhead were found

primarily to originate from Dworshak hatchery broodstock. These fish were also relatively old (2- and 3-ocean-age) and were derived from the Clearwater R., which is one of the regions expected to produce “B-run” steelhead. It is notable that the MGILCS reporting group represents some fish both within and outside the Snake River steelhead DPS, but does not represent all of the fish within the Snake River DPS.

This was the fifth year that we were able to analyze sockeye salmon using GSI, but for 2016 we used a GSI baseline that included kokanee samples from the Deschutes River drainage, Wallowa Lake, and other locations on the Snake River. We estimated relative stock composition and stock abundance for sockeye passing Bonneville Dam in 2016, and found that the Okanogan stock has the greatest relative abundance followed by the Wenatchee stock. We found fewer fish from the Snake River stock compared to previous years, and identified fish from Lake Billy Chinook and Wallowa Lake that previously would have assigned to the ‘Whatcom’ stock. We also found that the migratory run timing for the Okanogan and Wenatchee stocks overlaps broadly at Bonneville Dam, with the Snake and Lake Billy Chinook stocks migrating slightly later in the year.

References

- Hess, J.E., N.R. Campbell, A.P. Matala, S.R. Narum. 2012. 2011 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Hess, J.E., N.R. Campbell, A.P. Matala, S.R. Narum. 2013. 2012 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Steele et al. 2011.
- Steele CA, Campbell MR, Ackerman M, McCane J, Hess MA, Campbell N, Narum SR. 2011. Parentage Based Tagging of Snake River hatchery steelhead and Chinook salmon. Bonneville Power Administration. Annual Progress Report, Project number 2010-031-00.
- <https://research.idfg.idaho.gov/Fisheries%20Research%20Reports/Res11-111Steele2010%20Parentage%20Based%20Tagging%20Snake%20River%20Steelhead%20Salmon.pdf>

Overall Conclusion

This project combines four inter-related studies from the Fish & Wildlife Program Accords that address the following current and future objectives: 1) discover and evaluate SNP markers in salmon and steelhead and other anadromous fishes; 2) expand and create genetic baselines for multiple species including Chinook salmon, steelhead, sockeye salmon and kokanee, and coho salmon; 3) implement Genetic Stock Identification (GSI)/PBT programs for mainstem Chinook salmon, sockeye salmon, and steelhead fisheries and 4) GSI/PBT of fish passing Bonneville Dam (steelhead, sockeye, and Chinook salmon).

As described in Section 1, SNP panels continue to be expanded with GTseq that enables genotyping large sample sizes (>151,800 fish genotyped in 2017). This new genotyping protocol has greatly increased our laboratory's efficiency by allowing large numbers of fish to be genotyped with large numbers of SNP loci but at lower costs. For genetic baseline expansion (Objective 2), PBT hatcheries above Bonneville were genotyped to enable more thorough assignment of hatchery origin fish. In addition, GSI baselines are being developed to include two RAD-seq projects that have been initiated and will provide high density geographic coverage of Chinook salmon and steelhead populations in the Columbia River Basin. SNPs identified through these latter efforts will be useful in characterizing genetic diversity of hatchery and wild Chinook salmon and steelhead stocks. This study included two broad applications of stock identification; namely, stock composition of fisheries for Chinook salmon, sockeye salmon, and steelhead (Objective 3), and stock composition of Chinook salmon, sockeye salmon, and steelhead passing Bonneville Dam (Objective 4). Chinook salmon and steelhead fishery applications of GSI were integrated with the new genetic technology of parentage based tagging (PBT). The challenge imposed by long histories of exogenous stock transfers from specific hatchery programs often prevents effective application of GSI in assigning hatchery fish. However, as the role of PBT is expanding to tag all hatchery fish, the role of GSI will be focused on identifying stocks of natural-origin fish.

Our GSI analyses of harvest included stock composition results for the spring, summer, and fall management periods of Chinook salmon fisheries in the lower Columbia River mainstem.

Sockeye salmon and steelhead fisheries were analyzed and our stock composition results will provide additional information to managers of these fisheries. However, the sockeye salmon results indicate an increase in sample size may be warranted to make accurate estimates of rare stocks such as Snake River sockeye salmon. Although it was possible to estimate stock proportions of Snake River sockeye salmon, the low sample sizes precluded our ability to conclude whether there are significant differences in proportions of this stock among fisheries and at Bonneville Dam.

For Objective 4, we used a combination of GSI and PBT to estimate run-timing distributions and relative abundance of hatchery and wild Chinook salmon and steelhead stocks in 2016. For sockeye salmon, we used GSI to estimate relative stock abundance and run-timing distributions. The stock-specific data on abundance and run-timing of these species were used as a context for interpreting harvest stock composition.